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(54) Title: METHOD OF TREATMENT FOR LUNG DISEASES USING ANTISENSE OLIGONUCLEOTIDES

(57) Abstract

A method of treating airway disease in a subject in need of such treatment is disclosed. The method comprises topically administering to the subject an antisense oligonucleotide in an amount effective to treat the ariway disease, where the antisense oligonucleotide is essentially free of adenosine. Pharmaceutical formulations are also disclosed.

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METHOD OF TREATMENT FOR LUNG DISEASES USING ANTISENSE OLIGONUCLEOTIDES

This invention was made with Government support under grant RO1CA47217-06 from the National Cancer Institute. The Government has certain rights to this invention.

Field of the Invention

This application concerns a method of administering antisense oligonucleotides essentially free of adenosine as a treatment for lung diseases.

Background of the Invention

Antisense oligonucleotides have received considerable theoretical consideration as potentially useful pharmacologic agents in human disease. R. Wagner, *Nature* 372, 333-335 (1994). However, practical applications of these molecules in actual models of human disease have been elusive. One important consideration in the pharmacologic application of these molecules is route of administration. Most experiments utilizing antisense oligonucleotides *in vivo* have involved direct application to limited regions of the brain (see C. Wahlestedt, *Trends in Pharmacological Sciences* 15, 42-46 (1994); J. Lai et al., *Neuroreport* 5, 1049-1052 (1994); K. Standifer et al., *Neuron* 12, 805-810 (1994); A. Akabayashi et al., *Brain Research* 21, 55-61 (1994)), or to spinal fluid (see e.g. L. Tseng et al., *European J. Pharmacol.* 258, R1-3 (1994); R. Raffa et al., *European J. Pharmacol.* 258, R5-7 (1994); F. Gillardon et al., *European J. Neurosci.* 6, 880-884 (1994)). Such applications have limited clinical utility due to their invasive nature.

The systemic administration of antisense oligonucleotides also poses significant problems with respect to pharmacologic application, not the least of which is the difficulty in targeting disease-involved tissues. In contrast, the lung is an excellent potential target for antisense oligonucleotide application since it may be approached noninvasively and in a tissue-specific manner. Additionally, the lung represents an exceptional target for antisense ODN therapeutics ascompared to other *in vivo* target organs or tissues, possibly because the lung is lined with surfactant which consists primarily of cationic lipids, well known to enhance cellular uptake of ODNs in other systems. However, the technology involved in delivering antisense agents to the lung remains relatively undeveloped, and potential problems related to the application of antisense agents to the lung remain unexplored.

Adenosine, a purine which contributes to intermediary metabolism and participates in the regulation of physiological activity, is a recognized neuromodulator. This nucleoside is involved in many local regulatory mechanisms, in particular at synapses in the CNS and at neuroeffector junctions in the periphery. In the CNS adenosine is known to inhibit the release of a variety of neurotransmitters (noradrenaline, serotonin, GABA, acetylcholine, dopamine, glutamate, etc.), to inhibit neurotransmission, depress neuronal firing, induce spinal analgesia, and to possess anxiolytic properties (E.S. Ben-Soreket al., *Archives of Internal Medicine* **153**, 2701-2702 (1993)). In the heart, adenosine is known to slow atrioventricular (AV) conduction, suppress pacemaker activity, possess antiarrhythmic effects, modulate autonomic control, and to trigger the synthesis and release of prostaglandins. M.K. Church et al., *J. Allergy & Clinical Immunology* **92**, 190-194 (1993). It also possesses potent vasodilatory effects and modulates vascular tone. S.T. Holgate et al., *Annals*

of the New York Academy of Sciences **629**, 227-236 (1991).

As a therapeutic agent, adenosine has achieved considerable recent success as an antiarryhthmic agent in the treatment of supraventricular tachycardia. See C.G. DeGroff and M.J. Silka, *Journal of Pediatrics* **125**, 822-823 (1994); I. Drake et al., *Human and Exp. Toxicol.* **13**, 263-265 (1994). However, many adverse effects of adenosine treatment have been reported in the literature. See, e.g., A. Aggarwal, et al., *Anesthesiology* **79**, 1132-1135 (1993); K.K. Burkhart, *American J. Emergency Med.* **11**, 249-250 (1993); S.K. Srinivasan and P.J. Iversen, *J. Clin. Lab. Analysis* **9**, 129- 137 (1995); C.A. Stein et al., *Pharmacology & Therapeutics* **52**, 365-384 (1991); B.B. Fredholm et al., *Pharmacological Reviews* **46**, 143-156 (1994); H. Saito, et al., *Blood* **66**, 1233-1240 (1985). In particular, asthmatic individuals show an extreme sensitivity to adenosine and adenosine monophosphate. *See*, J.H. Butterfield et al., *Leukemia Res.* **12**, 345-355 (1988); *CLONETICS: Normal Human Cell Systems Manual* (1995); R.W. Wagner, *Nature* **372**, 333-335 (1994). Serious, near-fatal induction of bronchospasm has occurred in asthmatic individuals administered adenosine for supraventricular tachycardia. *See*, S. Tabor, in: *Current Protocols in Molecular Biology, Vol. 1*, Section 3.10.2 (John Wiley & Sons, 1987); J.H. Weiss, *Id.*, at Section 6.2.2.

Similarly, asthmatic rabbits produced using the dust mite allergic rabbit model of human asthma also were shown to respond to aerosolized adenosine with marked bronchoconstriction, while non asthmatic rabbits showed no response. S. Ali et al., *Agents Actions* 37, 165-176 (1992). Recent work using this model system has suggested that adenosine-induced bronchoconstriction and bronchial hyperresponsiveness in asthma are mediated primarily through the stimulation of adenosine receptors. S. Ali et

al., *J. Pharmacol. Exp. Ther.* 268, 1328-1334 (1994); S. Ali et al., *Am. J. Physiol* 266, L271-277 (1994).

Accordingly, adenosine is contraindicated in the lungs of asthmatics (who represent 10% of the adult and 15% of the pediatric population in the United States). Since antisense ODNs are typically composed of all four base pairs, adenine, guanine, cytosine and thymidine, their breakdown products will produce free deoxyadenosine monophosphate in these hyperresponsive airways. Deoxyadenosine monophosphate differs from adenosine monophosphate only by the loss of an oxygen atom on the 3' carbon of the sugar moiety.

Summary of the Invention

A first aspect of the present invention is a method of treating airway disease in a subject in need of such treatment. The method comprises administering an antisense oligonucleotide essentially free of adenosine to the lungs of the subject in an amount effective to treat the airway disease.

A second aspect of the present invention is a pharmaceutical composition, comprising, together in a pharmaceutically acceptable carrier, an antisense oligonucleotide essentially free of adenosine in an amount effective to treat an airway disease.

A third aspect of the present invention is the use of an antisense oligonucleotide essentially free of adenosine as given above for the preparation of a medicament for treating airway disease in a subject in need of such treatment.

Brief Description of the Drawings

Figures 1-4 demonstrate that antisense oligonucleotides can be utilized as effective agents in the treatment or prevention of airway diseases.

Figure 1 illustrates the effects of A_1 adenosine receptor antisense oligonucleotides and mismatch control

antisense oligonucleotides on the dynamic compliance of the bronchial airway in a rabbit model. **Figure 2** illustrates the specificity of A_1 adenosine receptor antisense oligonucleotides as indicated by the A_1 and A_2 adenosine receptor number present in A_1 adenosine receptor antisense oligonucleotide-treated airway tissue.

Figure 3 is a graphical representation illustrating that aerosolized deoxyadenosine monophosphate is a potent bronchoconstrictor in asthmatic pathways of allergic rabbits. Further, the figure shows that the effect of deoxyadenosine monophosphate is equipotent to that observed for adenosine monphosphate.

Figure 4 is a graphical representation illustrating that bronchoconstrictor effects occur with aerosolized phosphorothioate oligodeoxynucleotides containing adenosine, but not with oligodeoxynucleotides that are free of adenosine.

Detailed Description of the Invention

Nucleotide sequences are presented herein by single strand only, in the 5' to 3' direction, from left to right. Nucleotides and amino acids are represented herein in the manner recommended by the IUPAC-IUB Biochemical Nomenclature Commission, or (for amino acids) by three letter code, in accordance with 37 CFR §1.822 and established usage. *See, e.g., PatentIn User Manual*, 99-102 (Nov. 1990)(U.S. Patent and Trademark Office, Office of the Assistant Commissioner for Patents, Washington, D.C. 20231); U.S. Patent No. 4,871,670 to Hudson et al. at Col. 3 · lines 20-43 (applicants specifically intend that the disclosure of this and all other patent references cited herein be incorporated herein by reference).

The method of the present invention may be used to treat airway disease in a subject for any reason, with the intention that adenosine content of antisense compounds be eliminated or reduced so as to prevent its

liberation upon antisense degredation. Such liberation may cause serious, even life-threatening, bronchoconstriction in patients with hyperreactive airways. Examples of airway diseases that may be treated by the method of the present invention include cystic fibrosis, asthma, chronic obstructive pulmonary disease, bronchitis, and other airway diseases characterized by an inflammatory response.

Antisense oligonucleotides to the A_1 and A_3 receptors are shown to be effective in the downregulation of A_1 or A_3 in the cell. One novel feature of this treatment, as compared to traditional treatments for adenosine-induced bronchoconstriction, is that administration is direct to the lungs. Additionally, a receptor protein itself is reduced in amount, rather than merely interacting with a drug, and toxicity is reduced. Other proteins that may be targeted with antisense agents for the treatment of lung conditions include, but are not limited to: human A2a adenosine receptor, human A2b adenosine receptor, human IgE receptor β, human Fc-epsilon receptor CD23 antigen, human histidine decarboxylase, human beta tryptase, human tryptase-I, human prostaglandin D synthase, human cyclooxygenase-2, human eosinophil cationic protein, human eosinophil derived neurotoxin, human eosinophil peroxidase, human intercellular adhesion molecule-1 (ICAM-1), human vascular cell adhesion molecule 1 (VCAM-1), human endothelial leukocyte adhesion molecule (ELAM-1), human P selectin, human endothelial monocyte activating factor, human IL-3, human IL-4, human IL-5, human IL-6, human IL-8, human monocyte-derived neutrophil chemotactic factor, human neutrophil elastase, human neutrophil oxidase factor, human cathepsin G, human defensin 1, human defensin 3, human macrophage inflammatory protein-1-alpha, human muscarinic acetylcholine receptor HM1, human muscarinic acetylcholine receptor HM3, human fibronectin, human GM-CSF, human tumor necrosis factor α, human

leukotriene C4 synthase, human major basic protein, and human endothelin 1. In these latter targets, and in target genes in general, it is particularly imperative to eliminate or reduce the adenosine content of the corresponding antisense oligonucleotide to prevent their breakdown products from liberating adenosine.

As used herein, the term "treat" or "treating" a lung disease refers to a treatment which decreases the likelihood that the subject administered such treatment will manifest symptoms of the lung disease. The term "downregulate" refers to inducing a decrease in production, secretion or availability (and thus a decrease in concentration) of the targeted intracellular protein.

The present invention is concerned primarily with the treatment of human subjects but may also be employed for the treatment of other mammalian subjects, such as dogs and cats, for veterinary purposes. Targeted proteins are preferably mammalian and more preferably of the same species as the subject being treated.

In general, "antisense" refers to the use of small, synthetic oligonucleotides, resembling single-stranded DNA, to inhibit gene expression by inhibiting the function of the target messenger RNA (mRNA). Milligan, J.F. et al., *J. Med. Chem.* 36(14), 1923-1937 (1993). In the present invention, inhibition of gene expression of the A_1 or A_3 adenosine receptor is desired. Gene expression is inhibited through hybridization to coding (sense) sequences in a specific messenger RNA (mRNA) target by hydrogen bonding according to Watson-Crick base pairing rules. The mechanism of antisense inhibition is that the exogenously applied oligonucleotides decrease the mRNA or protein levels of the target gene or cause changes in the growth characteristics or shapes of the cells. *Id. See also* Helene, C. and Toulme, J., *Biochim. Biophys. Acta* 1049, 99-125 (1990); Cohen, J.S., Ed., *Oligodeoxynucleotides as*

Antisense Inhibitors of Gene Expression; CRC Press:Boca Raton, FL (1987).

As used herein, "antisense oligonucleotide" is defined as a short sequence of synthetic nucleotides that (1) hybridizes to any coding sequence in an mRNA which codes for the targeted protein, according to hybridization conditions described below, and (2) upon hybridization causes a decrease in gene expression of the targeted protein.

The mRNA sequence of the A_1 or A_3 adenosine receptor is derived from the DNA base sequence of the gene expressing either the A_1 or A_3 adenosine receptor. The sequence of the genomic human A_1 adenosine receptor is known and is disclosed in U.S. Patent No. 5,320,963 to G. Stiles et al. The A_3 adenosine receptor has been cloned, sequenced and expressed in rat (*see* F. Zhou et al., *Proc. Nat'l Acad. Sci. USA* **89**:7432 (1992)) and human (*see* M.A. Jacobson et al., U.K. Patent Application No. 9304582.1 (1993)). Thus, antisense oligonucleotides that downregulate the production of the A_1 or A_3 adenosine receptor may be produced in accordance with standard techniques.

One aspect of this invention is an antisense oligonucleotide having a sequence capable of binding specifically with any sequence of an mRNA molecule which encodes an airway disease-associated protein so as to prevent translation of the mRNA molecule.

Chemical analogs of oligonucleotides (e.g., oligonucleotides in which the phosphodiester bonds have been modified, e.g., to the methylphosphonate, the phosphotriester, the phosphorothioate, the phosphorodithioate, or the phosphoramidate, so as to render the oligonucleotide more stable *in vivo*) are also an aspect of the present invention. The naturally occurring phosphodiester linkages in oligonucleotides are susceptible to degradation by endogenously occurring cellular nucleases, while many analogous linkages are

highly resistant to nuclease degradation. *See* Milligan et al., and Cohen, J.S., *supra*. Protection from degradation can be achieved by use of a "3'-end cap" strategy by which nuclease-resistant linkages are substituted for phosphodiester linkages at the 3' end of the oligonucleotide. *See* Tidd, D.M. and Warenius, H.M., *Br. J. Cancer* 60, 343-350 (1989); Shaw, J.P. et al., *Nucleic Acids Res.* 19, 747-750 (1991). Phosphoramidates, phosphorothioates, and methylphosphonate linkages all function adequately in this manner. More extensive modification of the phosphodiester backbone has been shown to impart stability and may allow for enhanced affinity and increased cellular permeation of oligonucleotides. *See* Milligan, et al., *supra*. Many different chemical strategies have been employed to replace the entire phosphodiester backbone with novel linkages. *Id*. Backbone analogues include phosphorothioate, phosphorodithioate, methylphosphonate, phosphoramidate, boranophosphate, phosphotriester, formacetal, 3'-thioformacetal, 5'-thioformacetal, 5'-thioether, carbonate, 5'-N-carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, hydroxylamine, methylene(methylimino) (MMI) or methyleneoxy(methylimino) (MOMI) linkages. Phosphorothioate and methylphosphonate-modified oligonucleotides are particularly preferred due to their availability through automated oligonucleotide synthesis. *Id*. Where appropriate, the antisense oligonucleotides may be administered in the form of their pharmaceutically acceptable salts.

Antisense oligonucleotides may be of any suitable length (e.g., from about 10 to 60 nucleotides in length), depending on the particular target being bound and the mode of delivery thereof. Preferably the antisense oligonucleotide is directed to an mRNA region containing a junction between intron and exon. Where the antisense oligonucleotide is directed to an intron/exon

junction, it may either entirely overlie the junction or may be sufficiently close to the junction to inhibit splicing out of the intervening exon during processing of precursor mRNA to mature mRNA (e.g., with the 3' or 5' terminus of the antisense oligonucleotide being is positioned within about, for example, 10, 5, 3, or 2 nucleotides of the intron/exon junction).

When practicing the present invention, the antisense nucleotides administered may be related in origin to the species to which it is administered. When treating humans, human antisense may be used if desired.

Pharmaceutical compositions comprising an antisense oligonucleotide as given above effective to reduce expression of an A_1 or A_3 adenosine receptor by passing through a cell membrane and binding specifically with mRNA encoding an A_1 or A_3 adenosine receptor in the cell so as to prevent its translation are another aspect of the present invention. Such compositions are provided in a suitable pharmaceutically acceptable carrier (e.g., sterile pyrogen-free saline solution). The antisense oligonucleotides may be formulated with a hydrophobic carrier capable of passing through a cell membrane (e.g., in a liposome, with the liposomes carried in a pharmaceutically acceptable aqueous carrier). The oligonucleotides may also be coupled to a substance which inactivates mRNA, such as a ribozyme. Such oligonucleotides may be administered to a subject to inhibit the activation of A_1 or A_3 adenosine receptors, which subject is in need of such treatment for any of the reasons discussed herein. Furthermore, the pharmaceutical formulation may also contain chimeric molecules comprising antisense oligonucleotides attached to molecules which are known to be internalized by cells. These oligonucleotide conjugates utilize cellular uptake pathways to increase cellular concentrations of oligonucleotides. Examples of macromolecules used in this manner include transferrin, asialoglycoprotein

(bound to oligonucleotides via polylysine) and streptavidin.

In the pharmaceutical formulation the antisense compound may be contained within a lipid particle or vesicle, such as a liposome or microcrystal. The particles may be of any suitable structure, such as unilamellar or plurilamellar, so long as the antisense oligonucleotide is contained therein. Positively charged lipids such as N-[1-(2,3-dioleoyloxi)propyl]-N,N,N-trimethyl-ammoniumethylsulfate, or "DOTAP," are particularly preferred for such particles and vesicles. The preparation of such lipid particles is well known. *See, e.g.*, U.S. Patent Nos. 4,880,635 to Janoff et al.; 4,906,477 to Kurono et al.; 4,911,928 to Wallach; 4,917,951 to Wallach; 4,920,016 to Allen et al.;4,921,757 to Wheatley et al.; etc.

Subjects may be administered the active composition by any means which transports the antisense nucleotide composition to the lung. The antisense compounds disclosed herein may be administered to the lungs of a patient by any suitable means, but are preferably administered by generating an aerosol comprised of respirable particles, the respirable particles comprised of the antisense compound, which particles the subject inhales. The respirable particles may be liquid or solid. The particles may optionally contain other therapeutic ingredients.

Particles comprised of antisense compound for practicing the present invention should include particles of respirable size: that is, particles of a size sufficiently small to pass through the mouth and larynx upon inhalation and into the bronchi and alveoli of the lungs. In general, particles ranging from about .5 to 10 microns in size are respirable. Particles of non-respirable size which are included in the aerosol tend to deposit in the throat and be swallowed, and the quantity of non-respirable particles in the aerosol is preferably

minimized. For nasal administration, a particle size in the range of 10-500 μm is preferred to ensure retention in the nasal cavity.

Liquid pharmaceutical compositions of active compound for producing an aerosol can be prepared by combining the antisense compound with a suitable vehicle, such as sterile pyrogen free water. Other therapeutic compounds may optionally be included.

Solid particulate compositions containing respirable dry particles of micronized antisense compound may be prepared by grinding dry antisense compound with a mortar and pestle, and then passing the micronized composition through a 400 mesh screen to break up or separate out large agglomerates. A solid particulate composition comprised of the antisense compound may optionally contain a dispersant which serves to facilitate the formation of an aerosol. A suitable dispersant is lactose, which may be blended with the antisense compound in any suitable ratio (e.g., a 1 to 1 ratio by weight). Again, other therapeutic compounds may also be included.

The dosage of the antisense compound administered will depend upon the disease being treated, the condition of the subject, the particular formulation, the route of administration, the timing of administration to a subject, etc. In general, intracellular concentrations of the oligonucleotide of from .05 to 50 μM, or more particularly .2 to 5 μM, are desired. For administration to a subject such as a human, a dosage of from about .01, .1, or 1 mg/Kg up to 50, 100, or 150 mg/Kg or more is typically employed. Depending on the solubility of the particular formulation of active compound administered, the daily dose may be divided among one or several unit dose administrations. Administration of the antisense compounds may be carried out therapeutically (i.e., as a rescue treatment) or prophylactically.

Aerosols of liquid particles comprising the antisense compound may be produced by any suitable means, such as with a nebulizer. *See, e.g.*, U.S. Patent No. 4,501,729. Nebulizers are commercially available devices which transform solutions or suspensions of the active ingredient into a therapeutic aerosol mist either by means of acceleration of a compressed gas, typically air or oxygen, through a narrow venturi orifice or by means of ultrasonic agitation. Suitable formulations for use in nebulizers consist of the active ingredient in a liquid carrier, the active ingredient comprising up to 40% w/w of the formulation, but preferably less than 20% w/w. the carrier is typically water or a dilute aqueous alcoholic solution, preferably made isotonic with body fluids by the addition of, for example, sodium chloride. Optional additives include preservatives if the formulation is not prepared sterile, for example, methyl hydroxybenzoate, antioxidants, flavoring agents, volatile oils, buffering agents and surfactants.

Aerosols of solid particles comprising the active compound may likewise be produced with any solid particulate medicament aerosol generator. Aerosol generators for administering solid particulate medicaments to a subject produce particles which are respirable, as explained above, and generate a volume of aerosol containing a predetermined metered dose of a medicament at a rate suitable for human administration. One illustrative type of solid particulate aerosol generator is an insufflator. Suitable formulations for administration by insufflation include finely comminuted powders which may be delivered by means of an insufflator or taken into the nasal cavity in the manner of a snuff. In the insufflator, the powder (e.g., a metered dose thereof effective to carry out the treatments described herein) is contained in capsules or cartridges, typically made of gelatin or plastic, which are either pierced or opened *in situ* and the powder delivered by air drawn

through the device upon inhalation or by means of a manually-operated pump. The powder employed in the insufflator consists either solely of the active ingredient or of a powder blend comprising the active ingredient, a suitable powder diluent, such as lactose, and an optional surfactant. The active ingredient typically comprises from 0.1 to 100 w/w of the formulation. A second type of illustrative aerosol generator comprises a metered dose inhaler. Metered dose inhalers are pressurized aerosol dispensers, typically containing a suspension or solution formulation of the active ingredient in a liquified propellant. During use these devices discharge the formulation through a valve adapted to deliver a metered volume, typically from 10 to 150 μl, to produce a fine particle spray containing the active ingredient. Suitable propellants include certain chlorofluorocarbon compounds, for example, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane and mixtures thereof. The formulation may additionally contain one or more co-solvents, for example, ethanol, surfactants, such as oleic acid or sorbitan trioleate, antioxidants and suitable flavoring agents.

The aerosol, whether formed from solid or liquid particles, may be produced by the aerosol generator at a rate of from about 10 to 150 liters per minute, more preferably from about 30 to 150 liters per minute, and most preferably about 60 liters per minute. Aerosols containing greater amounts of medicament may be administered more rapidly.

The following examples are provided to illustrate the present invention, and should not be construed as limiting thereon. In these examples, μM means micromolar, mL means milliliters, μm means micrometers, mm means millimeters, cm means centimeters, °C means degrees Celsius, μg means micrograms, mg means

milligrams, g means grams, kg means kilograms, M means molar, and h means hours.

EXAMPLE 1

Design and synthesis of antisense oligonucleotides

The design of antisense oligonucleotides against the A_1 and A_3 adenosine receptors may require the solution of the complex secondary structure of the target A_1 receptor mRNA and the target A_3 receptor mRNA. After generating this structure, antisense nucleotides are designed which target regions of mRNA which might be construed to confer functional activity or stability to the mRNA and which optimally may overlap the initiation codon. Other target sites are readily usable. As a demonstration of specificity of the antisense effect, other oligonucleotides not totally complementary to the target mRNA, but containing identical nucleotide compositions on a w/w basis, are included as controls in antisense experiments.

Adenosine A_1 receptor mRNA secondary structure was analyzed and used as described above to design a phosphorothioate antisense oligonucleotide. The antisense oligonucleotide which was synthesized was designated **HAdA1AS** and had the following sequence:

5'-GAT GGA GGG CGG CAT GGC GGG-3' **(SEQ ID NO:1)**

As a control, a mismatched phosphorothioate antisense nucleotide designated **HAdA1MM** was synthesized with the following sequence:

5'-GTA GCA GGC GGG GAT GGG GGC-3' **(SEQ ID NO:2)**

Each oligonucleotide had identical base content and general sequence structure. Homology searches in GENBANK (release 85.0) and EMBL (release 40.0) indicated that the antisense oligonucleotide was specific for the human and rabbit adenosine A_1 receptor genes, and that the

mismatched control was not a candidate for hybridization with any known gene sequence.

Adenosine A_3 receptor mRNA secondary structure was similarly analyzed and used as described above to design two phosphorothioate antisense oligonucleotides. The first antisense oligonucleotide (**HAdA3AS1**) synthesized had the following sequence:

5'-GTT GTT GGG CAT CTT GCC-3' **(SEQ ID NO:3)**

As a control, a mismatched phosphorothioate antisense oligonucleotide (**HAdA3MM1**) was synthesized, having the following sequence:

5'-GTA CTT GCG GAT CTA GGC-3' **(SEQ ID NO:4)**

A second phosphorothioate antisense oligonucleotide (**HAdA3AS2**) was also designed and synthesized, having the following sequence:

5'-GTG GGC CTA GCT CTC GCC-3' **(SEQ ID NO:5)**

Its control oligonucleotide (**HAdA3MM2**) had the sequence:

5'-GTC GGG GTA CCT GTC GGC-3' **(SEQ ID NO:6)**

Phosphorothioate oligonucleotides were synthesized on an Applied Biosystems Model 396 Oligonucleotide Synthesizer, and purified using NENSORB chromatography (DuPont, MD).

EXAMPLE 2

Testing of A1-Adenosine Receptor Antisense Oligonucleotides *in vitro*

The antisense oligonucleotide against the human A_1 receptor (**SEQ ID NO:1**) described above was tested for

efficacy in an *in vitro* model utilizing lung adenocarcinoma cells HTB-54. HTB-54 lung adenocarcinoma cells were demonstrated to express the A_1 adenosine receptor using standard northern blotting procedures and receptor probes designed and synthesized in the laboratory.

HTB-54 human lung adenocarcinoma cells (106/100 mm tissue culture dish) were exposed to 5.0 μM **HAdA1AS** or **HAdA1MM** for 24 hours, with a fresh change of media and oligonucleotides after 12 hours of incubation. Following 24 hour exposure to the oligonucleotides, cells were harvested and their RNA extracted by standard procedures. A 21-mer probe corresponding to the region of mRNA targeted by the antisense (and therefore having the same sequence as the antisense, but not phosphorothioated) was synthesized and used to probe northern blots of RNA prepared from **HAdA1AS**-treated, **HAdA1MM**-treated and non-treated HTB-54 cells. These blots showed clearly that **HAdA1AS** but not **HAdA1MM** effectively reduced human adenosine receptor mRNA by >50%. This result showed that **HAdA1AS** is a good candidate for an anti-asthma drug since it depletes intracellular mRNA for the adenosine A_1 receptor, which is involved in asthma.

EXAMPLE 3

Efficacy of A_1-Adenosine Receptor Antisense Oligonucleotides *in vivo*

A fortuitous homology between the rabbit and human DNA sequences within the adenosine A_1 gene overlapping the initiation codon permitted the use of the phosphorothioate antisense oligonucleotides initially designed for use against the human adenosine A_1 receptor in a rabbit model.

Neonatal New Zealand white Pasteurella-free rabbits were immunized intraperitoneally within 24 hours of birth with 312 antigen units/mL house dustmite (*D. farinae*) extract (Berkeley Biologicals, Berkeley, CA),

mixed with 10% kaolin. Immunizations were repeated weekly for the first month and then biweekly for the next 2 months. At 3-4 months of age, eight sensitized rabbits were anesthetized and relaxed with a mixture of ketamine hydrochloride (44 mg/kg) and acepromazine maleate (0.4 mg/kg) administered intramuscularly.

The rabbits were then laid supine in a comfortable position on a small molded, padded animal board and intubated with a 4.0-mm intratracheal tube (Mallinkrodt, Inc., Glens Falls, NY). A polyethylene catheter of external diameter 2.4 mm with an attached latex balloon was passed into the esophagus and maintained at the same distance (approximately 16 cm) from the mouth throughout the experiments. The intratracheal tube was attached to a heated Fleisch pneumotachograph (size 00; DOM Medical, Richmond, VA), and flow was measured using a Validyne differential pressure transducer (Model DP-45161927; Validyne Engineering Corp., Northridge, CA) driven by a Gould carrier amplifier (Model 11-4113; Gould Electronic, Cleveland, OH). The esophageal balloon was attached to one side of the differential pressure transducer, and the outflow of the intratracheal tube was connected to the opposite side of the pressure transducer to allow recording of transpulmonary pressure. Flow was integrated to give a continuous tidal volume, and measurements of total lung resistance (RL) and dynamic compliance (Cdyn) were calculated at isovolumetric and flow zero points, respectively, using an automated respiratory analyzer (Model 6; Buxco, Sharon, CT).

Animals were randomized and on Day 1 pretreatment values for PC50 were obtained for aerosolized adenosine. Antisense (**HAdA1AS**) or mismatched control (**HAdA1MM**) oligonucleotides were dissolved in sterile physiological saline at a concentration of 5000 ug (5 mg) per 1.0 ml. Animals were subsequently administered the aerosolized antisense or mismatch

oligonucleotide via the intratracheal tube (approximately 5000 μg in a volume of 1.0 ml), twice daily for two days. Aerosols of either saline, adenosine, or antisense or mismatch oligonucleotides were generated by an ultrasonic nebulizer (DeVilbiliss, Somerset, PA), producing aerosol droplets 80% of which were smaller than 5 μm in diameter.

In the first arm of the experiment, four randomly selected allergic rabbits were administered antisense oligonucleotide and four the mismatched control oligonucleotide. On the morning of the third day, PC50 values (the concentration of aerosolized adenosine in mg/ml required to reduce the dynamic compliance of the bronchial airway 50% from the baseline value) were obtained and compared to PC50 values obtained for these animals prior to exposure to oligonucleotide.

Following a 1 week interval, animals were crossed over, with those previously administered mismatch control oligonucleotide now administered antisense oligonucleotide, and those previously treated with antisense oligonucleotide now administered mismatch control oligonucleotide. Treatment methods and measurements were identical to those employed in the first arm of the experiment. It should be noted that in six of the eight animals treated with antisense oligonucleotide, adenosine-induced bronchoconstriction could not be obtained up to the limit of solubility of adenosine, 20 mg/ml. For the purpose of calculation, PC50 values for these animals were set at 20 mg/ml. The values given therefore represent a minimum figure for antisense effectiveness. Actual effectiveness was higher. The results of this experiment are illustrated in both **Figure 1** and **Table 1**.

TABLE 1. EFFECTS OF ADENOSINE A_1 RECEPTOR ANTISENSE OLIGONUCLEOTIDE UPON PC50 VALUES IN ASTHMATIC RABBITS.

Mismatch Control		A_1 receptor Antisense oligonucleotide	
Pre oligonucleotide	Post oligonucleotide	Pre oligonucleotide	Post oligonucleotide
3.56 ± 1.02	5.16 ± 1.93	2.36 ± 0.68	>19.5 ± 0.34**

Results are presented as the mean (N = 8) ± SEM. Significance was determined by repeated-measures analysis of variance (ANOVA), and Tukey's protected t test. **Significantly different from all other groups, $P < 0.01$.

In both arms of the experiment, animals receiving the antisense oligonucleotide showed an order of magnitude increase in the dose of aerosolized adenosine required to reduce dynamic compliance of the lung by 50%. No effect of the mismatched control oligonucleotide upon PC50 values was observed. No toxicity was observed in any animal receiving either antisense or control inhaled oligonucleotide.

These results show clearly that the lung has exceptional potential as a target for antisense oligonucleotide-based therapeutic intervention in lung disease. They further show, in a model system which closely resembles human asthma, that downregulation of the adenosine A_1 receptor largely eliminates adenosine-induced bronchoconstriction in asthmatic airways. Bronchial hyperresponsiveness in the allergic rabbit model of human asthma is an excellent endpoint for antisense intervention since the tissues involved in this response lie near to the point of contact with aerosolized oligonucleotides, and the model closely simulates an important human disease.

EXAMPLE 4

Specificity of A_1-adenosine receptor Antisense oligonucleotide

At the conclusion of the crossover experiment of Example 3, airway muscle from all rabbits was quantitatively analyzed for adenosine A_1 receptor number. As a control for the specificity of the antisense oligonucleotide, adenosine A_2 receptors, which should not have been affected, were also quantified.

Airway smooth muscle tissue was dissected from each rabbit and a membrane fraction prepared according to described methods (J. Kleinstein and H. Glossmann, *Naunyn-Schmiedeberg's Arch. Pharmacol.* 305, 191-200 (1978), with slight modifications. Crude plasma membrane preparations were stored at - 70°C until the time of assay. Protein content was determined by the method of Bradford (M. Bradford, *Anal. Biochem.* 72, 240-254 (1976)). Frozen plasma membranes were thawed at room temperature and were incubated with 0.2 U/ml adenosine deaminase for 30 minutes at 37°C to remove endogenous adenosine. The binding of [^{3}H]DPCPX (A_1 receptor-specific) or [^{3}H]CGS-21680 (A_2 receptor-specific) was measured as previously described. S. Ali et al., *J. Pharmacol. Exp. Ther.* 268, 1328-1334 (1994); S. Ali et al., *Am. J. Physiol* 266, L271-277 (1994).

As illustrated in both **Figure 2** and **Table 2**, animals treated with adenosine A_1 antisense oligonucleotide in the crossover experiment had a nearly 75% decrease in A_1 receptor number compared to controls, as assayed by specific binding of the A_1-specific antagonist DPCPX. There was no change in adenosine A_2 receptor number, as assayed by specific binding of the A_2 receptor-specific agonist 2-[p-(2-carboxyethyl)-phenethylamino]-5'-(N-ethylcarboxamido) adenosine (CGS-21680).

TABLE 2. SPECIFICITY OF ACTION OF ADENOSINE A_1 RECEPTOR ANTISENSE OLIGONUCLEOTIDE.

	Mismatch Control oligonucleotide	A_1 Antisense oligonucleotide
A_1-Specific Binding	1105 ± 48**	293 ± 18
A_2-Specific Binding	302 ± 22	442 ± 171

Results are presented as the mean (N = 8) ± SEM. Significance was determined by repeated-measures analysis of variance (ANOVA), and Tukey's protected t test. **Significantly different from mismatch control, $P < 0.01$.

The above demonstrates the effectiveness of antisense oligonucleotides in treating airway diseases. Since the antisense oligonucleotides described above eliminate the receptor systems responsible for adenosine-mediated bronchoconstriction, it may be less imperative to eliminate adenosine from them. However, it would be preferable to eliminate adenosine from even these oligonucleotides. Examples of such adenosine-free oligonucleotides are provided below in Example 5.

EXAMPLE 5

The method of the present invention is also practiced with the following antisense oligonucleotides targeted to their corresponding proteins, in essentially the same manner as given above, for the treatment of various conditions in the lungs. Described below is a series of antisense oligonucleotides targetting the mRNA of proteins involved in inflammation. Adenosine has been eliminated from their nucleotide content to prevent its liberation during degradation.

In the following, the first sequence provided after the name of the targeted inflammation-involved protein is the antisense sequence that targets the initiation codon, wherein the naturally-occurring adenosine is substituted by one of the following: (1) a universal base that is not adenosine; (2) a adenosine analog that lacks the ability to bind to the adenosine A1

and/or A3 receptors; or (3) a "spacer." Any one of these three is represented in the sequence as the letter "B," recognized by the IUPAC-IUB Nomenclature Commission as "not-A." *See Patentin User Manual*, p.99 (November 1990). Listed following the antisense sequence targeted against the initation codon are additional antisense oligonucleotide sequences directed against other portions of the mRNA of the targeted protein. These additional sequences are the "*des*-adenosine antisense sequences," in that they do not contain adenosine within the sequence.

Fragments of the following sequences that are at least ten, and more preferably at least twelve, nucleotides in length are also an aspect of the presnet invention and are useful in carrying out the present invention. Fragments set forth below that span multiple lines of test indicate "5'-" at the beginning thereof, and "-3'" at the end thereof.

Human A1 adenosine receptor:

5'-GGC GGC CTG GBB BGC TGB GBT GGB GGG CGG CBT GGC GGG CBC BGG CTG GGC-3'

des-adenosine antisense sequences:

TTT TCC TTC CTT TGT CTC TCT TC

GCT CCC GGC TGC CTG

CTC GGC CGT GCG GCT CTG TCG CTC CCG GT

CCG CCG CCC TCC GGG GGG TC

TGC TGC CGT TGG CTG CCC

CTT CTG CGG GTC GCC GG

TGC TGG GCT TGT GGC

GGC CTC TCT TCT GGG

CCT GGT CCC TCC GT

GGT GGC TCC TCT GC

GCT TGG TCC TGG GGC TGC

TGC TCT CCT CTC CTT

Human A2a adenosine receptor:
GTBCBCCGBGGBGCCCBTGBTGGGCBTGCCBCBGBCGBCBGGC

des-adenosine antisense sequences:
HSA2ARECAS1: TGC TTT TCT TTT CTG GGC CTC **(SEQ ID NO:7)**
HSA2ARECAS2: TGT GGT CTG TTT TTT TCT G
HSA2ARECAS3: GCC CTG CTG GGG CGC TCT CC
HSA2ARECAS4: GCC GCC CGC CTG GCT CCC
HSA2ARECAS5: GGB GCC CBT GBT GGG CBT GCC
HSA2ARECAS6: GTG GTT CTT GCC CTC CTT TGG CTG
HSA2ARECAS7: CCG TGC CCG CTC CCC GGC
HSA2ARECAS8: CTC CTG GCG GGT GGC CGT TG
HSA2ARECAS9: GGC CCG TGT TCC CCT GGG
HSA2ARECAS10: GCC TGG GGC TCC CTT CTC TC
HSA2ARECAS11: GCC CTT CTT GCT GGG CCT C
HSA2ARECAS12: TGC TGC TGC TGG TGC TGT GGC CCCC

Human A2b adenosine receptor:
5'-BCBGCGCGTCCTGTGTCTCCBGCBGCBTGGCC
GGGCCBGCTGGGCCCC-3'

des-adenosine antisense sequences:
HSA2BRECAS1: 5'-GGC GCC GTG CCG CGT CTT GGT GGC GGC GG-3' **(SEQ ID NO:8)**
HSA2BRECAS2: 5'-GTT CGC GCC CGC GCG GGG CCC CTC CGG TCC-3'
HSA2BRECAS3: 5'-TTG GCC CGC GCG CCC GCC CGT CTC GGG CTG GGC GG-3'
HSA2BRECAS4: CGG GTC GGG GCC CCC CGC GGC C
HSA2BRECAS5: 5'-GCC TCG GGG CTG GGG CGC TGG TGG CCG GG-3'
HSA2BRECAS6: CCG CGC CTC CGC CTG CCG CTT CTG
HSA2BRECAS7: GCT GGG CCC CGG GCG CCC CCT
HSA2BRECAS8: CCC CTC TTG CTC GGG TCC CCG TG

Human A3 adenosine receptor
5'-BCB GBG CBG TGC TGT TGT TGG GCB TCT TGC CTT CCC BGG G-3'

des-adenosine antisense oligonucleotides:
CCC TTT TCT GGT GGG GTG

GTG CTG TTG TTG GGC

TTT CTT CTG TTC CC

Human IgE receptor β:
5'-BTTTGCTCTCCTBTTBCTTTCTGTGTCCBTTTTTT CBTTBBCCGBGCTGT-3'

des-adenosine antisense sequences:
HUMIgEβrAS1: TTT CCC CTG GGT CTT CC **(SEQ ID NO:9)**
HUMIgEβrAS2: CTC CTG CTC TTT TTT C

Human Fc-epsilon receptor CD23 antigen (IgE receptor):
5'-TCTCTGBBTBTTGBCCTTCCTCCBTGGCGGTCCTGCTT GGBTTCTCCCGB-3'

des-adenosine antisense sequences:
HUMIgErCD23AS1: GCC TGT GTC TGT CCT CCT **(SEQ ID NO:10)**
HUMIgErCD23AS2: GCT TCG TTC CTC TCG TTC
HUMIgErCD23AS3: CTG CTT GGT GCC CTT GCC G
HUMIgErCD23AS4: GTC CTG CTC CTC CGG GCT GTG G
HUMIgErCD23AS5: 5'-GTC GTG GCC CTG GCT CCG GCTGGT GGG CTC CCC TGG-3'
HUMIgErCD23AS6: CCT TCG CTG GCT GGC GGC GTG C
HUMIgErCD23AS7: GGG TCT TGC TCT GGG CCT GGC TGT
HUMIgErCD23AS8: GGC CGT GGT TGG GGG TCT TC
HUMIgErCD23AS9: GCT GCC TCC GTT TGG GTG GC

Human IgE receptor, α subunit:
5'-BCBGTBGBGTBGGGGBTTCCBTGGCBGGBGCCBTC TTCTTCBTGGBCTCC-3'

and

5'-TTC BBG GBG BCC TTB GGT TTC TGB GGG BCT GCT BBC BCG CCB TCT GGB GC-3'

des-adenosine antisense sequences:
HUMIgERαAS1: GCCTTTCCTGGTTCTCTT **(SEQ ID NO:11)**

GTT GTT TTT GGG GTT TGG CTT

Human IgE receptor, Fc epsilon R:
5'-GBT CTC TGB BTB TTGB CCT TCC BTG GCG GTC CTG CTT GGB-3'

des-adenosine antisense sequences:
HSJGEBFRAS1: GCC TGT GTC TGT CCT CCT **(SEQ ID NO:12)**
HSJGEBFRAS2: GCT TCG TTC CTC TCG TTC
HSJGEBFRAS3: CTG CTT GGT GCC CTT GCC G
HSJGEBFRAS4: GTC CTG CTC CTC CGG GCT GTG G
HSJGEBFRAS5: 5'-GTC CTC GCC CTG GCT CCG GCT GGT GGG CTC CCC TGG-3'
HSJGEBFRAS6: CCT TCG CTG GCT GGC GGC GTG C
HSJGEBFRAS7: CCC BGB BCG BGB CCC GGB CCG BCB
HSJGEBFRAS8: GGC CGT GGT TGG GGG TCT TC
HSJGEBFRAS9: GCT GCC TCC GTT TGG GTG GC

Human histidine decarboxylase:
5'-CTC TGT CCC TCT CTC TCT GTB CTC CTC BGG CTC CBT CBT CTC CCT TGG GC-3'

des-adenosine antisense sequences:

HUMHDCAS1: TCT CCC TTG GGC TCT GGC TCC TTC TC **(SEQ ID NO:13)**

HUMHDCAS2: TCT CTC TCC CTC TCT CTC TGT
HUMHDCAS3: CGCCTCCGCCCTGGCTGCTGGGGTGGTGGTGC
HUMHDCAS4: TTT TGT TCT TCC TTG CTG CC
HUMHDCAS5: GCC CCG CTG CTT GTC TTC CTC G

Human beta tryptase:

5'-GGG CCT GGC CTG GGG CBG GGG CCG CGT BGG CGC GGC TCG CCB GGB CGG GCB GCG CCB GCB GCB GCB GBT TCB GCB TCC TGG-3'

des-adenosine antisense sequences:

HUMBTRYPAS1: CTTGCTCCTGGGGGCCTCCTG **(SEQ ID NO:14)**
HUMBTRYPAS2: GTC CCT CCG GGT GTT CCC GGC

Human tryptase-I:

5'-CCT GGB CTG GGG CBG GGG CCG CGT BGG CGC GGC TCG CCB GGB CGG GCB GCG CCB GCB GCB GCB GGC TCB GCB TCC TGG CCB CGG BBT TCC-3'

des-adenosine antisense sequences:

HUMTRYAS1: CTTGCTCCTGGGGGCCTCCTG **(SEQ ID NO:15)**
HUMTRYAS2: GTC CCT CTG GCT G TT CCC GGC

Human prostaglandin D synthase:

5'-CCC CBG CBG GBC CBG TCC CBT CCB CBG CGT GTG BTG BGT BGC CBT TCT CCT GCB GCC GBG-3'

des-adenosine antisense sequences:

HUMPROSYNAS1:GGTGTGCGGGGCCTGGTGCC**(SEQ ID NO:16)**
HUMPROSYNAS 2: CCT GGG CCT CGG GTG CTG CCT GT
HUMPROSYNAS 3: GCG CTG CCT TCT TCT CCT GG
HUMPROSYNAS 4: 5'-GTC CTC GCC GGG GCC CTT GCT GCC CTG GCT GT -3'
HUMPROSYNAS 5: GCC CTG GGG GTC TGG GTT CGGCTGT

Human cyclooxygenase-2:

5'-TGB GCG CCB GGB CCG CGC BCB GCB GCB GGG CGC GGG CGB GCB TCG CBG CGG CGG GCB GGG-3'

des-adenosine antisense sequences:

HUMCYCLOXAS1: GGGCGCGGGCGBGCBTCGC**(SEQ ID NO:17)**
HUMCYCLOXAS2: TTT GGG CTT TTC TCC TTT GGT T

Human eosinophil cationic protein:

5'-CBG BCB BBT TTG GGB BGT GBB CBG TTT TGG BBC CBT GTT TCC CBG TCT CTG BGC TGT GGC-3'

des-adenosine antisense sequences:

HSECPAS1: CCTCCTTCC TGG TCT GTC TGC **(SEQ ID NO:18)**

Human eosinophil derived neurotoxin:

5'-CCC CBB CBG BBG BBG CBG BCB BBT TTG GGB BGT GBB CBG TTT TGG BBC CBT GTT TCC TGT-3'

des-adenosine antisense sequences:
HSEOSDNAS1: GCC CTG CTG CTC TTT CTG CT **(SEQ ID NO:19)**
HSEOSDNAS 2: TCC CTT GGT GGG TTG GGC C
HSEOSDNAS 3: GCT GGT TGT TCT GGG GTT C
HSEOSDNAS 4: TTG CTG CCC CTT CTG TCC C
HSEOSDNAS 5: TGT TTG CTG GTG TCT GCG C

Human eosinophil major basic protein:
GGG GGB GTT TCB TCT TGG CTT T

des-adenosine antisense sequences:
TCT CCC CTT GTT CCT CCC C

TCT CCT GCT CTG GTG TCT CCT C

TTC CCT CCC TCC CCT GCC

GTG TTG TCT GTG GGT GTC C

GTT TCG CTC TTG TTG CCC

TGG GCC CTT CCC TGC TGG

Human eosinophil peroxidase:
5'-GCB CCG TCC BGT GBT GGT GCG GTB CTT GTC GCT GCB GCG CTC GGC CTG GTC CCG GBG BGC-3'

des-adenosine antisense sequences:
HSEPAS1: GCGCTCGGCCTGGTCCCGG **(SEQ ID NO:20)**
HSEPAS2: GGG TCT CCT CTT GTT GTT GC
HSEPAS3: TTG CGC CTC CTG CTG GGG GT CC
HSEPAS4: CTC TGT TCT TGT TTT GGG GGC
HSEPAS5: GGG CCC GGC CGT TGT CTT G
HSEPAS6: GTT TGG GGG TTT CCG TTG
HSEPAS7: GGG TTC TCC TGG CCC GGG CCT TGC CC
HSEPAS8: GGC CGT GGT CCC GGC TTC GTT GC
HSEPAS9: CCT GTC TCC GTC TCG GCT CTT CTG
HSEPAS10: GGG CCT TGC GCT GTC TTT GGT G

Human intercellular adhesion molecule-1 (CAM-1):

5'- CGG BGC CTC CCC GGG GCB GGB TGB CTT TTG BGG GGG BCB CBG BTG TCT GGG CBT TGC CBG GTC CTG GGB BCB GBG CCC CGB GCB GGB CCB GGB GTG CGG GCB GCG CGG GCC GGG GGC TGC TGG GBG CCB TBG CGB GGC TGB G-3'

des-adenosine antisense sequences:
HSICAM1AS1: GCGCGGGCCGGGGGCTGCTGGG **(SEQ ID NO:21)**
HSICAM1AS2: GGT TGG CCC GGG GTG CCC C
HSICAM1AS3: GCC GCT GGG TGC CCT CGT CCTCTGCGGTC
HSICAM1AS4: GTG TCT CCT GGC TCT GGT TCC CC
HSICAM1AS5: 5'-GCT GCG CCC GTT GTC CTC TGG GGT GGCCTTC-3'

HSICAM1AS6: GCT CCC GGG TCT GGT TCT TGT GT
HSICAM1AS7: TGG GGG TCC CTT TTT GGG CCT GTT GT
HSICAM1AS8: GGC GTG GCT TGT GTG TTC GGT TTC
HSICAM1AS9: TGC CCT GTC CTC CGG CGT CCC

Human vascular cell adhesion molecule 1 (VCAM-1):
5'-CTG BGC BBG BTB TCT BGB TTC TGG GGT GGT CTC GBT TTT BBBB GCT TGB GBB GCT GCB BBC BTT BTC CBB BGT BTB TTT GBG GCT CCB BGG BTC BCG BCC BTC TTC CCB GGC BTT TTB BGT TGC TGT CGT -3'

des-adenosine antisense sequences:
HSVCAM1AS1: CCTCTTTTCTGTTTTTCCC **(SEQ ID NO:22)**
HSVCAM1AS2: CTC TGC CTT TGT TTG GGT TCG
HSVCAM1AS3: CTT CCT TTC TGC TTC TTC C
HSVCAM1AS4: CTGTGTCTCCTGTCTCCGCTTTTTTCTTC
HSVCAM1AS5: GTC TTT GTT GTT TTC TCT TCC TTG

Human endothelial leukocyte adhesion molecule (ELAM-1):
5'-BBG TGB GBG CTG BGB GBB BCT GTG BBG CBB TCB TGB CTT CBB GBG TTC TTT TCB CCC -3'

des-adenosine antisense sequences:
HUMELAM1AAS1: GTTCTTGGCTTCTTCTGTC **(SEQ ID NO:23)**
HUMELAM1AAS2: CGT TGG CTT CTC GTT GTC CC
HUMELAM1AAS3: TGT GGG CTT CTC GTT GTC CC
HUMELAM1AAS4: CCC TTC GGG GGC TGG TGG
HUMELAM1AAS5: GGC CGT CCT TGC CTG CTG G

Human P Selectin:
des-adenosine antisense sequences:
HUMPSELECTAS1: CTCTGCTGGT TTTCTGCCTT CTGCCC **(SEQ ID NO:24)**

Human endothelial monocyte activating factor:
des-adenosine antisense sequences:
HUMEMAPIIAS1: 5'-TTT TCT CTT TCG CTT TCT TTT CGTCTCCTGTTCCTCCTTTT-3' **(SEQ ID NO:25)**
HUMEMAPIIAS2: 5'-TTG CTG TTT TTT CTC CTT CTT CTC TCC TTT CTT TTC -3'

Human IL3:
5'-GGCGGBCCBGGBGTTGGBGCBGGBGCBGGBCGGGCB GGCGGCTCBTGTTTGGBTCGGCBGGBGGCBCTC -3'

des-adenosine antisense sequences:
HUMIL3AAS1: 5'-CTC TGT CTT GTT CTG GTC CTT CGT GGG GCT CTG **(SEQ ID NO:26)**-3'
HUMIL3AAS2: TGT CGC GTG G GTG CGG CCG TGG CC

Human IL3 receptor:
5'-GCBGGBGBCBGGGCBGGGCGBTCBGGBGCBGCGT GBGCCBBBGGBGGBCCBTCGGGBBCGCBGCTCCG GBBCGCBGGBCBGBGGTGCC-3'

des-adenosine antisense sequences:
TCTGGGGTGTCCTG

GCCTTCGTGGTTCC

TCTTCCTTCGTTTGC

CGTCCGCGGGGGCCCCCGGGCCT

GGCTGCGCTCCTGCCCCGC

CTCTTTCCCGGGCTCTT

GCGCTGGGGGGTGCTCC

CGTGTGTTTGCGCCCTCCTCCTGGTCGC

GCTTGTCGTTTTGG

GGCCGGCTTTGCCCGCCTCCC

GGCGCCTGGCCCGGCC

TTCCTGGGCTGCGTGCGC

GTTCTGTTCTTCTTCCTGGC

Human IL4:
5'-GCCGGCBCBTGCTBGCBGGBBGBBCBGBGGGGGB
BGCBGTTGGGBGGTGBGBCCCBTTBBTBGGTGTCGB-3'

des-adenosine antisense sequences:
HUMIL4AS1: CTC TGG TTG GCT TCC TTC-3'
(SEQ ID NO:27)

Human IL4 receptor:
5'-GTTCCCBGBGCTTGCCBCCTGCBGCBGGBCCBGGCBGCTC
BCBGGGBBCBGGBGCCCBGBGCBBBGCCBCCCCBTTGGGBG
BTGCCBBGGCBCCBGGCTG-3'

des-adenosine antisense sequences:
TCTGCGCGCCCCTGCTCC

CGCCCGGCTTCTCT

CGTGTGGGCTTCGG

CCCCGCGCCTCCGTTGTTCTC

TGCTCGCTGGGCTTG

GGTTTCCTGGGGCCCTGGGTTTC

TCTGCCGGGTCGTTTTC

GGGTGCTGGCTGCG

CTTGGTGCTGGGGCTCC

GGCGGCTGCGGGCTGGGTTGGG

CTTGGCTGGTTCCTGGCCTCGGG

CCTCCTCCTCCTCCTC

GCTCCCTTTTTCTTCCTCT

TCCCTGCTGCTCTC

TGCCCTCCCTTCCCTCCTGG

GGTGCCTCCTTGGGCCCTGC

GGCTGCTCCTTGCCCC

CTCTGGGTCGGGCTGGC

GGGGCGTCTCTGTGC

CTGGCCTGGGTGCC

GCCTCTCCTGGGGG

GGTGGCTCCCTGTCC

CCTTTTCCCCCGGCTCC

GTGGGGGCTTTGGC

GGGGGTCTGTGGCCTGCTCCTGGGG

AGGGGTCTGGGGCCCTC

TTTTGGGGGTCTGGCTTG

GCCTGGCTGCCTTCC

GGGGCCTGCCGTGGGGC

TGTCCTCTGTTGCTCCCCTT

TGCCTGCTGTCTGG

GGTTCCCGCCTTCCCT

Human IL5:

5'-GTGGGBBTTTCTGTGGGGBTGGCBTBCBCGTBGGCB
GCTCCBBGBGCTBGCBBBCTCBBBTGCBGBBGCBTC
CTCBTGGCTCTGBBBCG -3'

des-adenosine antisense sequences:
HUMIL5AS1: TCC CTG TTT CCC CCC TTT **(SEQ ID NO:28)**
HUMIL5AS2: CGT TCT GCG TTT GCC TTT GGC
HUMIL5AS3: GTT TTT TGT TTG TTT TCT
HUMIL5AS4: CTC TCC GTC TTT CTT CTC C
HUMIL5AS5: CCT CCT GCC TGT GTC CCT GCT CCC C
HUMIL5AS6: GAG GGT TTC TGG CTT CCT CTC T
HUMIL5AS7: TGT CTC TCT GTC CTT TTG TT
HUMIL5AS8: 5'-TGT TGT GCG GCC TGG TGC TGC CCT GCCCCG GG-3'

Human IL5 receptor antisense oligonucleotide
5'-CTCBGTGGCCCCCBBBBGGBT
GBGTBBTBCBTGCGCCBCGBT
GBTCBTBTCCTTTTTBCTBTGBGG-3'

des-adenosine antisense sequences:
CCGTGTCTGTCGTGTCT

TTCCTTTGCTCTTG

GTGTGTCTTTGCTGT

GCCCTGCCTCTCTGC

Human IL6:
5'-CTCCTGGGGGTBCTGGGGCBGGGBB
GGCBGCBGGCBBCBCCBGGBGCBGC
CCCBGGGBGBBGGCBBCTGGBCCGB
BGGCGCTTGTGGBGBBGGBGTTCBT
BGCTGGGCTCCTGGBGGGGBGBTBGBGC-3'

des-adenosine antisense sequence:
HUMIL6AS1: GCT TCT CTT TCG TTC CCG GTG GGC TCG **(SEQ ID NO:29)**
HUMIL6AS2: GTG GCT GTC TGT GTG GGG CGG CT
HUMIL6AS3: GTG CCT CTT TGC TGC TTT C
HUMIL6AS4: GAT TCT TTG CCT TTT TCT GC

Human IL6 receptor antisense oligonucleotides
5'-GCBCGCCTCTTGCCBCCTCCTGCGCBGGGCB
GCGCCTTGGGGCCBGCGCCGCTCCCGGCGCG
GCCBGCBGGGCBGCCBGCBGCGCGCBGCCGB
CGGCCBGCBTGCTTCCTCCTCGGCTBCCBCT
CCBTGGTCCCGCBGBGGCGGBCBGGC-3'

des-adenosine antisense sequences:
GGGGGTGGCTTCCTGCC

GCGTCTCTGGGCCGTCCC

GTCCCTCGGCCCCGCGCCGCGCTCGGCTCCTCTCCC

TCTGGCCCGGCTC

GGGGCGGGGCGGGGCGGTGGGCGGGC

GGCGCTGCCCTGCGC

GCGGCGCTGGCCCC

TGCTGGCCGTCGGCTGCGCGCTGCTGGCTGCCCT

GCTGGCCGCGCCGGG

GCCTGTCCGCCTCTGCGGG

CGCTGTCTCCTGGC

TTGTCTTCCGGCTCT

TCTGCTGGGGTGGG

GCTGGGCGGCCGGCCCGGT

GCTGGGGCTCCTCGGGGGG

GGGGGCTCTTCCGG

GCTGTCTCCCTCCGGG

GCGGGGGTTTCTGGCC

GTGGGGGTCTTGCC

TGGCCTCCGGGCTCC

TGCTTGTCTTGCCTTCCTTC

TCTGGTCGGTTGTGGCTCG

GGGCTCCGTGGGTCCCTGGC

GCCCGTTTGTGTTTTGTC

TTTTCCCCTGGCGT

CCCTGTGCCCCTCTCCTCTCCTTCCTCTGCTTCTC

GCTCTCCTTTGTGGG

GCCCTCCCTGCTGCT

CTTGGTTTTGGGCT

TTTTTTCTCTTCCTCCTTTTTC

GTGCGTGGGCCTCC

Human monocyte-derived neutrophil chemotactic factor:

5'-GGGGTGGBBBGGTTTGGBGTBTGTCTTTBTGCBCTGB
CBTCTBBGTTCTTTBGCBCTCCTTGGCBBBBCTGCBC
CTTCBCBCBGBGCTGCBGBBBTCBGGBBGGCTGCCBB
GBGBGCCBCGGCCBGCTTGGBBGTCBTGTTTBCBCBC
BGTGBGBTGGTTCCTTCCGG-3'

des-adenosine antisense sequences:

HSMDNCFAS1: GCT TGT GTG CTC TGC TGT CTC T **(SEQ ID NO:30)**
HSMDNCFAS2: 5'-TGG TTC CTT CCG GTG GTT TCT TCC TGG CTC TTG TCC T -3'
HSMDNCFAS3: TTC TCT TGG CCC TTG GC

Human neutrophil elastase (medullasin):

5'-GGGCTCCCGCCGCGBGBGGTTBTGGGCTCCCBGGBCCBC
CCGCBCCGCGCGGBCGTTTBCBTTCGCCBCGCBGTGCGC
GGCCGBCBTGBCGBBGTTGGGCGCBBTCBGGGTGGCGCC
GCBGBBGTGGCCTCCGCGCBGCTGCBGGGBCBCCBTGBB
GGGCCBCGCGTGGGGCCGCGCTCGCCGGCCCCCCBCBBT
CTCCGBGGCCBGCGCGGTGCCCCCCBGCBGCBBGGCCGG
CBGGBCBCBGGCGBGGBGBCBCGCGBGTCGGCGGCCGBG
GGTCBTGGTGGGGCTGGGGCTCCGGGGTCTCTGCCCCTC
CGTGC-3'

des-adenosine antisense oligonucleotides:

HSMEDURAS1: 5'-TGG TGG GGC TGG GGC TCC GGG GTC TCT GCC CCT CCG TGC-3' **(SEQ ID NO:31)**
HSMEDURAS2: CGC GTG GGG CCG CGC TCG CCG GCCCCCC
HSMEDURAS3: CCT GCC GGG TGG GCT CCC GCC GCG
HSMEDURAS4: CGC CGG CCT GCC GGC CCC TC
HSMEDURAS5: 5'-GTG GGT CCT GCT GGC CGG GTC CGG GTC CCG GGG GTG GGG-3'
HSMEDURAS6: CGC GBG TCG GCG GCC GBG GGT C

Human neutrophil oxidase factor:

5'-CGGGBGTGGGGGTCCTGGBCGGCBCTGBBGGCBTCCBGGG
CTCCCTTCCBGTCCTTCTTGTCCGCTGCCBGCBCCCCTTC
BTTCCBGBGGCTGBTGGCCTCCBCCBGGGBCBTGBTTBGG
TBGBBBCTBGGBGGCC-3'

des-adenosine antisense sequence:

HUMNOXFAS1: GGC CTC CBC CBG GGB CBT G **(SEQ ID NO:32)**
HUMNOXFAS2: GTC CTT CTT GTC CGC TGC C
HUMNOXFAS3: TCT CTG GGG TTT TCG GTC TGG GTG G
HUMNOXFAS4: GCT TTC CTC CTG GGG CTG CTG CTG
HUMNOXFAS5: 5'-GGC TCT TCT TTT TGT TTC TGG CCT GGTG-3'
HUMNOXFAS6: CTC TCT CGT GCC CTT TCC
HUMNOXFAS7: CTT GGG TGT CTT GTT TTT GT
HUMNOXFAS8: 5'-GGCCTCCBCCBGGGBCBTGGTCCTTCTT GTCCGCTGCC -3'

Human cathepsin G:

5'-CCCTCCBCBTCTGCTCTGBCCTGCTGGBCTCTG
GBTCTGBBGBTBCGCCBTGTBGGGGCGGGBGTG
GGGCCTGCTCTCCCGGCCTCCGBTGBTCTCCCCT
GCCTCBGCCCCBGTGGGTBGGBGBBBGGCCBGCB
GBBGCBGGBGTGGCTGCBTCTTTCCTG -3'

des-adenosine antisense sequences:

HUMCTHGAS1: GTG GGG CCT GCT CTC CCG GCC TCC G **(SEQ ID NO:33)**

HUMCTHGAS2: TGTGTTGCTGGGTGTTTTCCCGTCTCTGG

HUMCTHGAS3: TCT GCC TTC GGG GGT CGT

Human defensin 1:

5'-CCGGGGCTGCBGCBBCCTCBTCBGCTCTTGCCT
GGBGTGGCTCBGCCTGGGCCTGCBGGGCCBCCB
GGBGBBTGGCBGCBBGGBTGGCGBGGGTCCTCB
TGGCTGGGGTCBCBGBTCCTCTBGCTBGGCBGG
GTGBCCBGBGBGGGC-3'

des-adenosine antisense sequences:

HUMDEF1AAAS1: GGG TCC TCB TGG CTG GGG **(SEQ ID NO:34)**

HUMDEF1AAAS2: GCC TGG GCC TGC BGG GCC

HUMDEF1AAAS3: GCT CTT GCC TGG BGT GGC TC

HUMDEF1AAAS4: GCC CBG BGT CTT CCC TGG T

Human defensin 3:

5'-CGCTGCBBTCTGCTCCGGGGCTGCBGCBBCCTCBTC
BGCTCTTGCCTGGBGTGGCTCBGCCTGGGCCTGCBG
GGCCBCCBGGBGBBTGGCBGCBBGGBTGGCGBGGGT
CCTCBTGGCTGGGGTCBCCTGGBGGBGGGBGBGCBGG-3'

des-adenosine antisense sequences:

HUMNTRIIIAS1: GGG TCC TCB TGG CTG GGG TC **(SEQ ID NO:35)**

HUMNTRIIIAS2: CCT CTC TCC CGT CCT

Human macrophage inflammatory protein-1-alpha: RANTES RECEPTOR

5'-GBGGGGGCBGCBGTTGGGCCCCBBBGGCCCTCTCGT
TCBCCTTCTGGCBCGGBGTTGCBTCCCCBTBGTCBB
BCTCTGTGGTCGTGTCBTBGTCCTCTGTGGTGTTTG
GBGTTTCCBTCCCGGCTTCTCTCTGGTTCCBBGGGB-3'

des-adenosine antisense sequences:

HUMRANTESAS1: GTC TTT GTT TCT GGG CTC GTG CC **(SEQ ID NO:36)**

HUMRANTESAS2: CCB TCC CGG CTT CTC TCT GGT TCC

HUMRANTESAS3: GTC CTCTGT GGT GTT TGG

HUMRANTESAS4: 5'-CCC TGC TTC CTT TTG CCT GTT TCTTTGTTT CTGGGCTCGT GCC -3'

RANTES:

5'-GGGCBCGGGGCBGTGGGCGGGCBBTGTBGGC
BBBGCBGCBGGGTGTGGTGTCCGBGGBBTBTGGG
GBGGCBGBTGCBGGBGCGCBGBGGGCBGTBGCBB
TGBGGBTGBCBGCGBGGCGTGCCGCGGBGBCCTTC
BTGGTBCCTGTGGBGBGGCTGTCGGBGG-3'

des-adenosine antisense sequences:

GGGTGTGGTGTCCG

CTTGGCGGTTCTTTCGGGTG

TTTCTTCTCTGGGTTGGC

CTGCTGCTCGTCGTGGTC

GCTCCGCTCCCGGGTTC

GTCTCGCTCTGTCGCCC

CTTCCTTCCTTGTC

GTGTTCCTCCCTTCCTTGCCTCT

Human muscarinic acetylcholine receptor HM1:

des-adenosine antisense sequences:
HSHM1AS1: GTT CBT GGT GGC TBG GTG GGG C **(SEQ ID NO:37)**
HSHM1AS2: GCT GCC CGG CGG GGT GTG CGC TTG GC
HSHM1AS3: GCTCCCGTG CTC GGT TCT CTG TCTCCCGGT
HSHM1AS4: CCC CCT TTG CCT GGC GTC TCG G
HSHM1AS5: GCC TTC GTC CTC TTC CTC TTC TTC CTTCC
HSHM1AS6: 5'-GCT CCG TGG GGG CTG CTTGGTGGG GGCCTG TGC CTC GGG GTC C-3'
HSHM1AS7: CGG GGC TTC TGG CCC TTG CC

Human muscarinic acetylcholine receptor HM3:

des-adenosine antisense sequences:
HSHM3AS1: GGG GTG GGT BGG CCG TGT CTG GGG **(SEQ ID NO:38)**
HSHM3AS2: GTT GGC CBT GTT GGT TGC C
HSHM3AS3: TCT TGG TGG TGC GCC GGG C
HSHM3AS4: 5'-GCG TCT TGG CTT TCT TCT CCT TCG GGC CCT CGG GCC GGT GCT TGT GG-3'
HSHM3AS5: 5'-GCT CCT CCC GGG CGG CCT CCC CGG GCG GGG GCT TCT TG-3'
HSHM3AS6: GCG CTG GCG GGG GGG CCT CCT CC
HSHM3AS7: 5'-GCT CTG TGG CTG GGC GTT CCT TGG TGT TCT GGG TGG C-3'
HSHM3AS8: TGG CGG GCG TGG TGG CCT CTG TGG TGG
HSHM3AS9: GGG CCC GCG GCT GCB GGG G
HSHM3AS10: TTG CCT GTC TGC TTC GTC
HSHM3AS11: CTT TGC GCT CCC GGG CCG CC

Human fibronectin:

des-adenosine antisense sequences:

HUMFNA/HSFIB1AS1: CGG TTT CCT TTG CGG TC **(SEQ ID NO:39)**
HUMFNA/HSFIB1AS2: TTG GCC CGG GCT CCG GGT G
HUMFNA/HSFIB1AS3: CCC GCC CGC CCG CCG GCC GCCGC
HUMFNA/HSFIB1AS4: 5'-CCC GCC GGG CTG TCC CCG CCC CGC CCC-3'
HUMFNA/HSFIB1AS5: GGC CCG GGG CGC GGG GG
HUMFNA/HSFIB1AS6: CGG CCC TCC CGC CCC TCT GG
HUMFNA/HSFIB1AS7: GCC GGC GCG GGC GTC GG
HUMFNA/HSFIB1AS9: 5'-CCG CTC GCG CCT GGG GTT CCC TCT CCT CCCCCTGTGC-3'
HUMFNA/HSFIB1AS10: GCC TGC CTC TTG CTC TTC
HUMFNA/HSFIB1AS11: TGC GTC CGC TGC CTT CTC CC
HUMFNA/HSFIB1AS12: CTC TCC TCG GCC GTT GCCTGTGC
HUMFNA/HSFIB1AS13: 5'-TGT CCG TCC TGT CGC CCT TCC GTG GTG C-3'
HUMFNA/HSFIB1AS14: TGT TGT CTC TTC TGC CCT C
HUMFNA/HSFIB1AS15: GGT GTG CTG GTG CTGGTGGTGGTG
HUMFNA/HSFIB1AS16: CCT CTG CCC GTG CTC GCC
HUMFNA/HSFIB1AS17: CTG CCT GGG CTG GCCTCTTCGGGT
HUMFNA/HSFIB1AS18: 5'-GTG GCT TTG GGG CTC TCT TGG TTG CCC TTT-3'
HUMFNA/HSFIB1AS19: 5'-CTT CTC GTG GTG CCT CTC CTC CCT GGC TTG GTC GT-3'
HUMFNA/HSFIB1AS20: TGT CTG GGG TGG TGCTCCTCTCCC
HUMFNA/HSFIB1AS21: TTT CCC TGC TGG CCG TTT GT
HUMFNA/HSFIB1AS22: CCT GTT TTC TGT CTT CCT CT
HUMFNA/HSFIB1AS23: TTC CTC CTG TTT CTC CGT
HUMFNA/HSFIB1AS24: 5'-TTG GCT TGC TGC TTG CGG GGC TGT CTC C-3'
HUMFNA/HSFIB1AS25: CTT GCC CCT GTG GGC TTT CCC
HUMFNA/HSFIB1AS26: TGG TCC GGT CTTCTCCTTGGGGGTC
HUMFNA/HSFIB1AS27: GCC CTT CTT GGT GGG CTG
HUMFNA/HSFIB1AS28: GCT CGT CTG TCT TTT TCC TTCC
HUMFNA/HSFIB1AS29: 5'-TGG GGG TGG CCG TTG TGG GCG GTG TGG TCC GCC T-3'
HUMFNA/HSFIB1AS30: TGC CTC TGC TGG TCT TTC

Human interleukin 8:

5'-GBTGTTTGTTBCCBBBGCBTCBBGBBTBGCTTTGC
TBTCTBBGGBTCBCBTTTBGBCBTBGGBBBBCGC
TGTBGGTCBGBBBGBTGTGCTTBCCTTCBCBCBG
BGCTGCBGBBBTCBGGBBGGCTGCCBBGBGBGCC
BCGGCCBGCTTGGBGTCBTGTTTBCBCBCBGTGBG-3'

des-adenosine antisense sequences:

HUMIL8AAS1: GTG CTC CGG TGG CTT TTT **(SEQ ID NO:40)**
HUMIL8AAS2: GCT TGT GTG CTC TGC TGT CTC TG
HUMIL8AAS3: 5'-TTC CTT CCG GTG GTT TCT TCC TGG CTC TTG TCC T-3'
HUMIL8AAS4: TTC TCT TGG CCC TTG GCC C

Human IL-8 receptor-alpha

5'-BCBGGGGCTGTBBTCTTCBTCTGCBGGTGGCB
TGCCBGTGBBBTTTBGBTCBTCBBBBTCCCBCBT
CTGTGGBTCTGTBBTBTTTGBCBTGTCCTCTTC
BGTTTCBGCBBTGGTTTGBTCTBBCTGBBGCBCCG
GCCBGG-3'

des-adenosine antisense sequences:

TGGCTCGGTGCTTCTGCCCC

TGTTGTTGCGGCGCTC

GGTTGGTGTGGCCCCTG

TGGTGCTTCGTTTCC

CCCTCTTTCTCTTTGTTC

GGGGGTTCTTGTGGC

GGGCTGCTTGTCTCGTTCC

Human GM-CSF:

5'-CTTGBGCBGGBBGCTCTGGGGCBGGGBGCTGGCBG
GGCCCBGGGGGGTGGCTTCCTGCBCTGTCCBGBGT
GCBCTGTGCCBCBGCBGCBGCTGCBGGGCCBTCBG
CTTCBTGGGGCTCTGGGTGGCBGGTCCBGCCBTGG
GTCTGGGTGGGGCTGGGCTGCBGGCTCCGGGC-3'

des-adenosine antisense sequences:

HUMGCSFAS1:GGT CCB GCC BTG GGT CTG GG **(SEQ ID NO:41)**
HUMGCSFAS2:GGC TGG GCT GCB GGC TCC GG
HUMGCSFAS3: GCG GGC GGG TGC GGG CTG CGT GCT GGG
HUMGCSFAS4: GGC TGC CCC GCA GGC CCT GC

Human tumor necrosis factor α:

5'-CBCCGCCTGGBGCCCTGGGGCCCCCCTGTCTTCTTGGG
GBGCGCCTCCTCGGCCBGCTCCBCGTCCCGGBTCBTGCTTT
CBGTGCTCBTGGTGTCCTTTCCBGGGGBGBGBGGG-3'

des-adenosine antisense sequences

HSTNFAAS1: GCT GGT CCT CTG CTG TCC TTG CTG **(SEQ ID NO:42)**
HSTNFAAS2: GTG CTC BTG GTG TCC TTT CC
HSTNFAAS3: GCC CTG GGG CCC CCC TGT CTT CTT GGGG
HSTNFAAS4: CCT CTT CCC TCT GGG GGC CG
HSTNFAAS5: TCT CTC TCC CTC TCT TGC GTC TCT C
HSTNFAAS6: TCT TTC TCT CTC TCT CTT CCC C
HSTNFAAS7: TTT CCC GCT CTT TCT GTC TC
HSTNFAAS8: GGT GTC TGG TTT TCT CTC TCC
HSTNFAAS9: GCT GGC TGC CTG TCT GGC CTG CGC TCTT
HSTNFAAS10: GGC CTG TGC TGT TCC TCC
HSTNFAAS11: TCC GGT TCC TGT CCT CTC TGT CTG TC
HSTNFAAS12: GCC CCC TCT GGG GTC TCC CTC TGG C
HSTNFAAS13: GTG GTG GTC TTG TTG CTT

HSTNFAAS14: GGG CTG GGC TCC GTG TCT C
HSTNFAAS15: CBG TGC TCB TGG TGT CC
HSTNFAAS16: GCT GBG GGB GCG TCT GCT GGC

Human leukotriene C4 synthase:

5'-CTCGGTBGBCGCGCTCGBBCTCGGGTGGGCCGGTGGTG
BGCGGCGGCGBCBCGCGGBBGGCCCTGCGCGCCGBGBTCBC
CTGCBGGGBGBBGTBGGCTTGCBGCBGGBCTCCCBGGBGGG
TGBCBGCBGCCBGTBGBGCTBCCTCGTCCTTCBTGGTBCCG
TCGGTGTGGTGGCBCGGGCTGTGTGTGBBGGCGBGCTGG-3'

des-adenosine antisense sequences:

HSU11552AS1:GCC CCG TCT GCT GCT CCT CGT GCC G
(SEQ ID NO:43)
HSU11552AS2: 5'-CCT CGT CCT TCA TGG TAC CGT CGGTGT GGT GGC-3'
HSU11552AS3: CTC GGG TGG GCC GGT GGT G
HSU11552AS4: GGG CGC GCG CGC TCG CGT
HSU11552AS5: 5'-GGC TCC GGC TCT TCT TTC CCG GCTCCG TCG GCC CGG GGG CCTTGGTCTC-3'
HSU11551AS6:CCT CGT CCT TCB TGG TBC CG

Human Endothelin-1:

5'-BCCGGCGGBGCCGCCBGGGTGGBCTGGGBGTGGGTT
TCTCCCCGCCGTTCTCBCCCBCCGCGCTGBGCTCBGCGC
CTBBGBCTGCTGTTTCTGGBGCTCCTTGGCBBGCCBCBB
BCBGCBGBGBGBBBBTCBTGBGCBBBTBBTCCBTTCTGB
BBBBBBGGGBTCBBBBBCCTCCCGT-3'

des-adenosine antisense sequences:

CCCGTTCGCCTGGCGC

GCGCTGCGGGTTCCTC

GTGGGTTTCTCCCCGCCGTTCTC

CGGTCTGTTGCCTTTGTGGG

CTTCTTGTCTTTTTGGCT

GTTCTTTTCCTGCTTGGC

GTCTTTTCCTTTCTT

TGTGCTCGGTTGTGGGTC

CGCTGGTCCTTTGCC

CTGTGTGTTTCTGCTG

Endothelin receptor ET-B antisense oligonucleotides

5'-GCCCTGTCGGGCGGGBBGCCTCTCTCCTCTCCCCBG
BTCCGCGBCBGGCCGCBGGCBBGBBCCBGCGCBBCCBGG
GCGCGTCCGCBCBGBCTTGGBGGCGGCTGCBTGCTGCTB
CCTGCTCCBGBBGCGTCCGGTGGCCGCCGC-3'

des-adenosine antisense sequences:
GCGTCCGGTGGCCGCCGC

GCCTCTCTCCTCTCCCC

GTGGCCCTGTCGGGCGGG

TCCTGCCGTCCTGTCTCCTTT

TCTTTTGCTGTCTTGT

CTTCCCGTCTCTGCTTT

Endothelin ETA receptor antisense oligonucleotides
5'-CBTCCBCBTGBTTGCTTBGBTTTGTGCTGTBTCTCTCB
GGBTTBTCBCTGBTTBCBCBTCCBBCCBGTGCCBGCCBBBB
GGBTGCCCTGBGGCBBBGGGTTTCCBTCTTGBGGCBBBTTT
GBGGB-3'

des-adenosine antisense sequences:
GTCTGTCCTCCCCGTCTCCTCCC

ACTGCTTCTCCCGGGG

GCTTCCCCGGCTTC

GGGTGGCCGGTGTCCCGGGCTCCGGCGCGGCGGC

GGCTTCGGCTGC

GGGTGGGTGGCGCGG

GCTGCCGGGTCCGCGCGGCGCCTGGGCC

CTTGTGCTGCTTTT

TGCTTGTTCCGTTC

TGGCTGCTCCGGTCTGTGTTGTGGTTGTTTTG

TTTCTTCTTGGGTGTGGG

CCTTGCGGTTTTGG

CTGTGGGCCCTTTG

GGGCCTTGGCTTCTGGCTC

Substance P antisense oligonucleotide
5'-CTGCTGBGGCTTGGGTCTCCGGGCGBTTCTCTGCBGBBGBT
GCTCBBBGGGCTCCGGCBGTTCCTCCTTGBTCTGGTCGCTGTCG
TBCCBGTCGGBCCBGTBBTTCBGBTCBTCBTTGGCTCCTBTTTC
TTCTGCBBBCBGCTGBGTGGBGBCBBGBBBBBBGBCTGCCBBGG
CCBCGBGGBTTTTCBTGTTGGBTTTTGCGBCGGBCBGTCCCGCG
GGGTGCTGAGTTTCTCTGGTTCCTCCGBGCGCB-3'

des-adenosine antisense sequences:
CGTGGTCGCTCCGC

TTTCTCTGGTTCCTCCG

GTCCCGCGGGGTGCTG

TCTGGTCGCTGTCGT

GGCTTGGGTCTCCGGGCG

GTTTCCTTCCTTTTCCGC

Substance P receptor antisense oligonucleotide
5'-GGCTBBGBTGBTCCBCBTCBCTBCCBCGTTGCCCBCCBCB
GBGGTCBCCBCBBTGBCCGTGTBGGCBGCTGCCCBBBGGBCBB
TTTGCCBGGCTGGTTGCBCGBBCTGBTTGGGTTCCGBGGTGTT
BGTGGBGBTGTTTGGGGBGBGGTCTGBGTCCBCCGGGBGGBCG
TTBTCCBTTTCGBBGCTBGGCGGTBBBGCCCTBCTBTCTGTBC
BCBBCCCCCCTCTGCBGCBGBGTCCTGTCGTGGCGCCTGGGGC
TCBGGGTCC-3'

des-adenosine antisense sequences:
GTCCTGTCGTGGCGCCTGGGGCTC

TTCTTTTGTGGGCT

CTTTGGTGGCTGTGGCTG

TGGTCTCTGTGGTTG

CTGCCCTGGGTCTGG

GGGTGTGGCCTTGGGGCCGTCCTCTGGCTCCTCCTCGTGGGCCCCC

Chymase
5'-GGBGCTGBTBCTGCBGATTTCBGBGGGBBGBBCCCT
GBTBCTCBCCBGCTTCBGCTCTGGBGCBCBBGBGBBBGB
GCBGCBGGGGGBGBGGBBGBBGCBGCBTCTTCCCBGBGB
GGCTGCCTGBGCBBBTGCTGGTTTTCCTTTCCBGTCTTG
GGTTTTBTBBCTCCCBGBBGGCBBGBGBGGGGCBBGG-3'

des-adenosine antisense sequences:
CGTTTTCTTCTCTC

TGCTGGTTTTCCTTTCC

TGGCAGTGGGTGGGGGTGGGGGTGGGGTGGC

TTCCTTGTTCCTGGGGGTGTCCT

CTTGCTCTGGGCTTTTCT

CCCCTTTTCCTTCC

TGTCTGTTTTCCTGGGG

CTCTCCTCTGTCTCTGTGT

CCTTGCCCTGGCCC

TCTTCCCTCTCCTGTCTCCTGT

CCCTGTGTTCCGCCC

GTCTTCCCTCTCCTG

ACCTCCTTTTCCTCCG

CTGGGTGGGGCCCTG

CCTGTTCTCTGCTCCC

TGGCTTGGGGTTTCTTCTG

TGTGTCTTCTTCCTCTGTT

GGCTGGCTTTCTCCTTC

TTTTGTCTTCCTGGG

TGCCCCTTCTTCCTTTCTTGGG

TCCTTGGTGCTTGGGCTGGG

Endothelial nitric oxide synthase

5'-GCGTCTTGGGGTGCBGGGCCCBTCCTGCTGCGCCTGGGCG
CTGBGGGTGTCBTBGGTGBTGCTCCCCBCCTCCCBGTTCTTCB
CBCGBGGGBBCTTGGGCCCCTCTGGGGGCTGGGTTBGCGGGGB
GCTCGGGGGGCTGTGTTCTGGCGCTGGTGGGBGTBGGGBTGCT
GGGGCCCGGCTGGGCTCBGGGGCCGGGGTGGCTGGGCCCTGCT
TGCCGCBCBGCCCBBGGCCCBGCCCCBGCCCCBGGCCGCBGGG
TGGCCCBGGCTCCTGGGCCBCGCTCTTCBBGTTGCCCBTGTTB
CTGTGCGTCCGTCTGCTGGBGCBGGCBGCBGBGTGGGBBTTC-3'

des-adenosine antisense sequences:

CTGTGCGTCCGTCTGCTGG

GGGGCCGGGGTGGCTGGGCCCTGCTTGCCGC

ACGACCCCGGGCCGACCCGAG

GCTCGGGGGGCTGTGTTCTGGCGCTGGTGGG

CTTGGGCCCCTCTGGGGGCTGGGTT

TCCTGCTGCGCCTGGGCGCTG

GCGTCTTGGGGTGC

GGGGCCGGGGGGCCGGGGG

GCCGCTGTTCGTGGGCCTGGG

GGTGCCTGTGGCTGCC

GGTTGCCCCGGTTGGTGGC

GCCGTCCTGCTGCCGGT

CGTTGGCTGGGTCCCCCCGC

CCGTTTCCTGGGGTCC

GCGTGGGGTGCTCC

GGTTCCTCGTGCCG

CTGCTGCCTTGTCTTTCC

GGCCGTGGCGGCGTGGTGGTCC

GCCCCCCCTGGCCTTCTGCTC

GGGGTCTGGCTGGT

TGCCGGTGCCCTTGGCGGC

GGTCTTCTTCCTGGTG

GCTCTGGGCCCGGCCGGTCTCGG

GCGTCTCGTGTTCG

CTCTTGTGCTGTTCCGGCCG

CTCCTTCCTCTTCCGCCGCC

GCCGCTCCCCGCCC

GCTCGTCGCCCTGGCCC

GGCCTCCTCCTGGCCGC

TGTCTCGGGCGGCGGCCTTGGC

GCTCCGTTTGGGGCTG

CCTCTGGCGCTTCC

GGCCCTCGGCCTGGGCGCTC

TCTTCCGCCTGTGC

TGGTGGCCCTCGTGG

GCCCCTCCTGGCCTCCGGTGTCC

TGTGGTCCCCCGGCTGGT

GGCCGGGCCGGTTGGGCGGGC

GTGGGCGCCGGCGGGTCCTCC

GGGCTGCCCTTCTCC

GCCGGGGGTCCCGC

GCTCCTGCTGTTCCCTGGGCTCTTCTGCC

TCTCTCCTGGGTGGGTGCTGGGTGCCG

GGGTCTCCGGGCTTG

CCCCGCGCTGCTGGGCGTTCTGC

GGTCTTGGGGTTGTC

TGTGGCCCCGCTCG

TGTCGCCCTCCGTCGCC

CGTCGCCGGCCTCGTCC

CCTCCTGGGTGCGC

GGCGGGCTGGTCCT

GGCGTTTTGCTCCTTCCTGG

Inducible nitric oxide synthase

5'-CTGCCCCBGTTTTTGBTCCTCBCBTGCCGTGGGGBGGB
CBBTGGGGTTGCBTCCBGCTTGBCCBGBGBTTCTGGBG
BCTTCTTTCCCGTCTCCBCGBGGGGCTGCGGGGBCTCB
TTCTGCTGCTTGCTGBGGTTGTGBTBCTGBGGTCBTCC
TGTGTCBCTGGBCTGGBGGTGGCBCBGGGGGCTTTCTC
CBCBTTGTTGTTGBTGTCTTTTTCCCCBTTCBTTGCBT
BCTGGTGGBBTTTGGTCTTGBBCBGBBBTTTCCBBGGB
CBGGCCBTCTCTBTGGCTTTBCBBBGCBGGTCBCTTBT
GTCBCTTBTCTGGBTTTGBGCTCBGBTGTTCTTCBCTG
TGGGGCTTGCBGCTGGCTGCBCTGCCTCCCCGGGGTB-3'

Human major basic protein:

GTTTCATCTT GGCTTTATCC **(SEQ ID NO:44)**

EXAMPLE 6

Turning now to **Figure 3**, two asthmatic rabbits were adminstered adenosine, and two rabbits were adminstered dAMP, at the indicated concentrations, by inhalation as described above in Example 3. The results (shown in **Figure 3** as change in compliance) indicate that dAMP, a breakdown product of antisense

oligodeoxynucleotides containing adenosine, is as potent in the induction of bronchoconstriction as adenosine in the hyperresponsive airways of asthmatic rabbits.

EXAMPLE 7

An aerosolized phosphorothioate 21-mer antisense ODN consisting of 50% adenosine and 50% guanine plus cytosine in a random configuation was found to produce potent bronchoconstrictor effects in hyperreactive airways of asthmatic rabbits, as illustrated in **Figure 4**. The control molecule used in this study, a phosphorothioate 21-mer antisense ODN consisting of 50% guanine and 50% thymidine plus cytosine (*des*-adenosine ODN) produced no bronchoconstrictor or any other effect in these same animals.

In this study, bronchoconstrictor effects were measured as a percentage change in bronchial compliance. Each group consisted of two allergic rabbits, and data shown are for the period following the second of two daily administrations of 5 mg aerosolized ODN by nebulizer.

These results indicate that antisense oligonucleotides, even when modified to slow degradation, produce adenosine metabolites capable of potent bronchoconstriction when adminstered in asthmatic airways.

The foregoing examples are illustrative of the present invention, and are not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Nyce, Jonathan W.

(ii) TITLE OF INVENTION: Method of Treatment of Lung Diseases Using Antisense Oligonucleotides

(iii) NUMBER OF SEQUENCES: 44

(iv) CORRESPONDENCE ADDRESS:
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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Sibley, Kenneth D.
(B) REGISTRATION NUMBER: 31,665
(C) REFERENCE/DOCKET NUMBER: 5218-32

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (919) 881-3140
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(C) TELEX: 575102

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GATGGAGGGC GGCATGGCGG G 21

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GTAGCAGGCG GGGATGGGGG C 21

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTTGTTGGGC ATCTTGCC 18

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GTACTTGCGG ATCTAGGC 18

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GTGGGCCTAG CTCTCGCC 18

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GTCGGGGTAC CTGTCGGC 18

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TGCTTTTCTT TTCTGGGCCT C 21

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGCGCCGTGC CGCGTCTTGG TGGCGGCGG 29

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TTTCCCCTGG GTCTTCC 17

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GCCTGTGTCT CTCCTCCT 18

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GCCTTTCCTG GTTCTCTT 18

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GCCTGTGTCT GTCCTCCT 18

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TCTCCCTTGG GCTCTGGCTC CTTCTC 26

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CTTGCTCCTG GGGGCCTCCT G 21

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CTTGCTCCTG GGGGCCTCCT G 21

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GGTGTGCGGG GCCTGGTGCC 20

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 12
(D) OTHER INFORMATION: /standard_name= "Reduced A"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 15
(D) OTHER INFORMATION: /standard_name= "Reduced A"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GGGCGCGGGC GAGCATCGC 19

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CCTCCTTCCT GGTCTGTCTG C 21

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GCCCTGCTGC TCTTTCTGCT 20

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GCGCTCGGCC TGGTCCCGG 19

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GCGCGGGCCG GGGGCTGCTG GG 22

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CCTCTTTTCT GTTTTTCCC 19

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GTTCTTGGCT TCTTCTGTC 19

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CTCTGCTGGT TTTCTGCCTT CTGCCC 26

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TTTTCTCTTT CGCTTTCTTT TCGTCTCCTG TTCCTCCTTT T 41

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CTCTGTCTTG TTCTGGTCCT TCGTGGGGCT CTG 33

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CTCTGGTTGG CTTCCTTC 18

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

TCCCTGTTTC CCCCCTTT 18

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GCTTCTCTTT CGTTCCCGGT GGGCTCG 27

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GCTTGTGTGC TCTGCTGTCT CT 22

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

TGGTGGGGCT GGGGCTCCGG GGTCTCTGCC CCTCCGTGC 39

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GTCCTTCTTG TCCGCTGCC 19

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GTGGGGCCTG CTCTCCCGGC CTCCG 25

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GGGTCCTCAT GGCTGGGG 18

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 9
(D) OTHER INFORMATION: /standard_name= "Reduced A"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GGGTCCTCAT GGCTGGGGTC 20

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GTCTTTGTTT CTGGGCTCGT GCC 23

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 5
(D) OTHER INFORMATION: /standard_name= "Reduced A"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 14
(D) OTHER INFORMATION: /standard_name= "Redcued A"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GTTCATGGTG GCTAGGTGGG GC 22

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 10
(D) OTHER INFORMATION: /standard_name= "Reduced A"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GGGGTGGGTA GGCCGTGTCT GGGG 24

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

CGGTTTCCTT TGCGGTC 17

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

GTGCTCCGGT GGCTTTTT 18

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 6
(D) OTHER INFORMATION: /standard_name= "Reduced A"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 10
(D) OTHER INFORMATION: /standard_name= "Reduced A"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

GGTCCAGCCA TGGGTCTGGG 20

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

GCTGGTCCTC TGCTGTCCTT GCTG 24

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

GCCCCGTCTG CTGCTCCTCG TGCCG 25

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 6
(D) OTHER INFORMATION: /standard_name= "Reduced A"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 17
(D) OTHER INFORMATION: /standard_name= "Reduced A"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

GTTTCATCTT GGCTTTATCC 20

THAT WHICH IS CLAIMED IS:

1. A method of treating airway disease in a subject in need of such treatment, comprising:

topically administering an antisense oligonucleotide to the airway epithelium of said subject in an amount effective to treat said disease;

said antisense oligonucleotide being essentially free of adenosine.

2. A method according to claim 1 wherein said airway disease is a lung disease and said airway epithelium is a lung airway epithelium.

3. A method according to claim 1 wherein said antisense oligonucleotide comprises nucleotides in which at least one phosphodiester linkage is replaced with a linkage selected from the group consisting of methylphosphonate linkages, phosphotriester linkages, phosphorothioate linkages, phosphorodithioate linkages, and phosphoramidate linkages.

4. A method according to claim 1 wherein said airway disease is selected from the group consisting of cystic fibrosis, asthma, chronic obstructive pulmonary disease, bronchitis, and other airway diseases characterized by an inflammatory response.

5. A method according to claim 1 wherein said antisense oligonucleotide is targeted against an mRNA encoding a protein selected from the group consisting of human A2a adenosine receptor, human A2b adenosine receptor, human IgE receptor β, human Fc-epsilon receptor CD23 antigen, human histidine decarboxylase, human beta tryptase, human tryptase-I, human prostaglandin D synthase, human cyclooxygenase-2, human eosinophil cationic protein, human eosinophil derived neurotoxin, human eosinophil peroxidase, human intercellular adhesion

molecule-1 (ICAM-1), human vascular cell adhesion molecule 1 (VCAM-1), human endothelial leukocyte adhesion molecule (ELAM-1), human P selectin, human endothelial monocyte activating factor, human IL-3, human IL-4, human IL-5, human IL-6, human IL-8, human monocyte-derived neutrophil chemotactic factor, human neutrophil elastase, human neutrophil oxidase factor, human cathepsin G, human defensin 1, human defensin 3, human macrophage inflammatory protein-1-alpha, human muscarinic acetylcholine receptor HM1, human muscarinic acetylcholine receptor HM3, human fibronectin, human GM-CSF, human tumor necrosis factor α, human leukotriene C4 synthase, human major basic protein, and endothelin 1.

6. A method according to claim 1 wherein said antisense oligonucleotide is delivered by administering an aerosol of respirable particles containing said antisense oligonucleotide to the lungs of said subject.

7. A method according to claim 6, wherein said particles are selected from the group consisting of solid particles and liquid particles.

8. A method according to claim 6, wherein said aerosol is comprised of particles having a particle size within the range of about 0.5 to 10 microns.

9. A method according to claim 8 wherein said particles are liposomes containing said antisense oligonucleotide.

10. A method according to claim 6 wherein said antisense oligonucleotide is administered in amount sufficient to achieve intracellular concentrations of said antisense oligonucleotide in said subject from about 0.1 to 10 μM.

11. A pharmaceutical composition, comprising, together in a pharmaceutically acceptable carrier:

an antisense oligonucleotide in an amount effective to treat an airway disease;

said antisense oligonucleotide being essentially free of adenosine.

12. A pharmaceutical composition according to claim 11 wherein said airway disease is a lung disease and said airway epithelium is a lung airway epithelium.

13. A pharmaceutical composition according to claim 11 wherein said antisense oligonucleotide comprises nucleotides in which at least one phosphodiester linkage is replaced with a linkage selected from the group consisting of methylphosphonate linkages, phosphotriester linkages, phosphorothioate linkages, phosphorodithioate linkages, and phosphoramidate linkages.

14. A pharmaceutical composition according to claim 11 wherein said airway disease is cystic fibrosis.

15. A pharmaceutical composition according to claim 11 wherein said antisense oligonucleotide is targeted against an mRNA encoding a protein selected from the group consisting of human A2a adenosine receptor, human A2b adenosine receptor, human IgE receptor β, human Fc-epsilon receptor CD23 antigen, human histidine decarboxylase, human beta tryptase, human tryptase-I, human prostaglandin D synthase, human cyclooxygenase-2, human eosinophil cationic protein, human eosinophil derived neurotoxin, human eosinophil peroxidase, human intercellular adhesion molecule-1 (ICAM-1), human vascular cell adhesion molecule 1 (VCAM-1), human endothelial leukocyte adhesion molecule (ELAM-1), human P selectin, human endothelial monocyte activating factor, human IL-3, human IL-4, human IL-5, human IL-6, human IL-

8, human monocyte-derived neutrophil chemotactic factor, human neutrophil elastase, human neutrophil oxidase factor, human cathepsin G, human defensin 1, human defensin 3, human macrophage inflammatory protein-1-alpha, human muscarinic acetylcholine receptor HM1, human muscarinic acetylcholine receptor HM3, human fibronectin, human GM-CSF, human tumor necrosis factor α, human leukotriene C4 synthase, and human major basic protein.

16. A pharmaceutical composition according to claim 11 wherein said antisense oligonucleotide is delivered by administering an aerosol of respirable particles containing said antisense oligonucleotide to the lungs of said subject.

17. A pharmaceutical composition according to claim 16, wherein said particles are selected from the group consisting of solid particles and liquid particles.

18. A pharmaceutical composition according to claim 16, wherein said aerosol is comprised of particles having a particle size within the range of about 0.5 to 10 microns.

19. A pharmaceutical composition according to claim 16 wherein said particles are liposomes containing said antisense oligonucleotide.

20. A pharmaceutical composition according to claim 11 wherein said antisense oligonucleotide is administered in amount sufficient to achieve intracellular concentrations of said antisense oligonucleotide in said subject from about 0.1 to 10 μM.

21. A pharmaceutical composition according to claim 11, wherein said antisense oligonucleotide is conjugated to a molecule capable of cellular uptake.

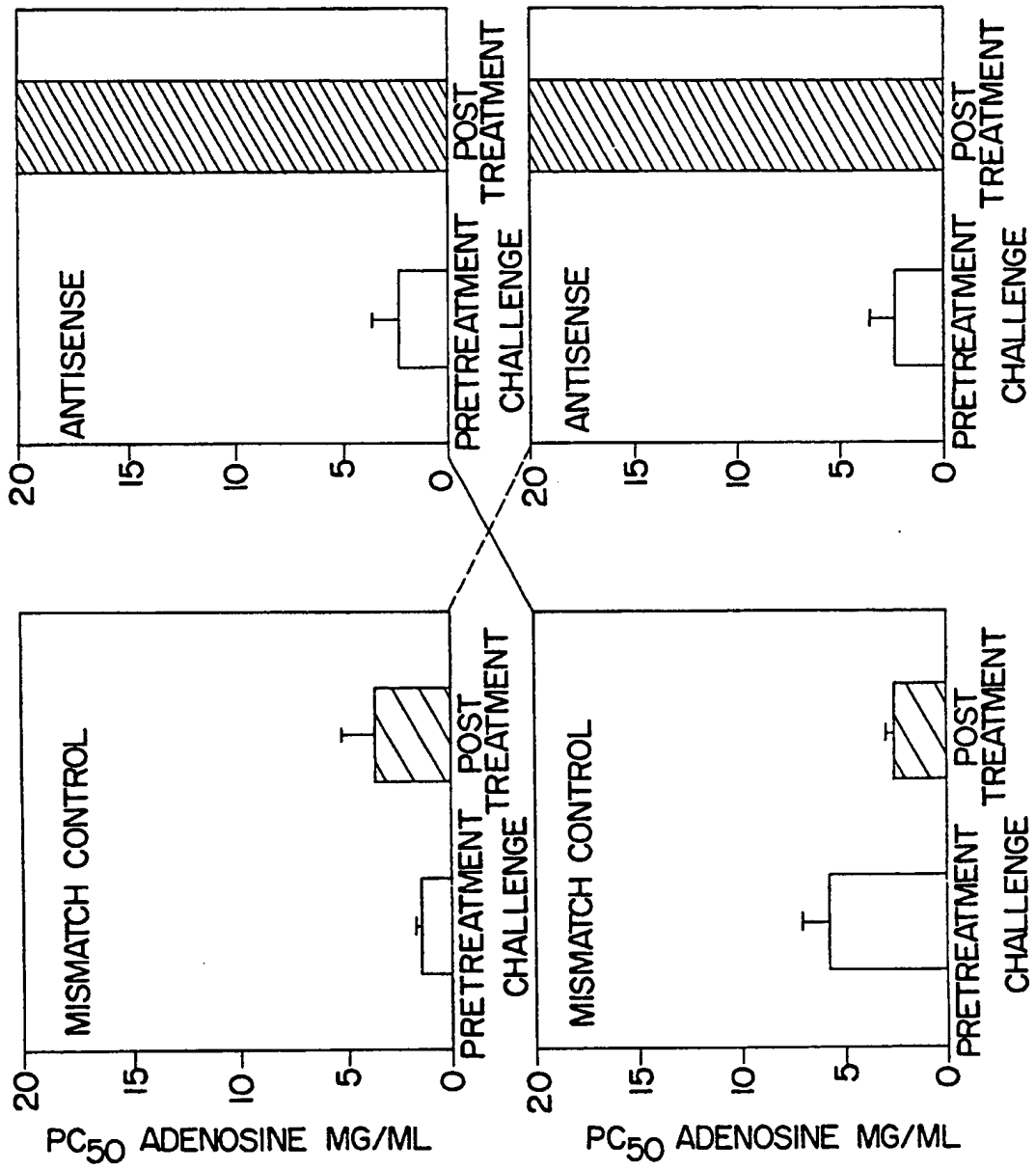
ANTISENSE
MISMATCH CONTROL
PRETREATMENT
CHALLENGE
POST TREATMENT
20
15
10
5
0
PC50 ADENOSINE MG/ML

FIG. 1.

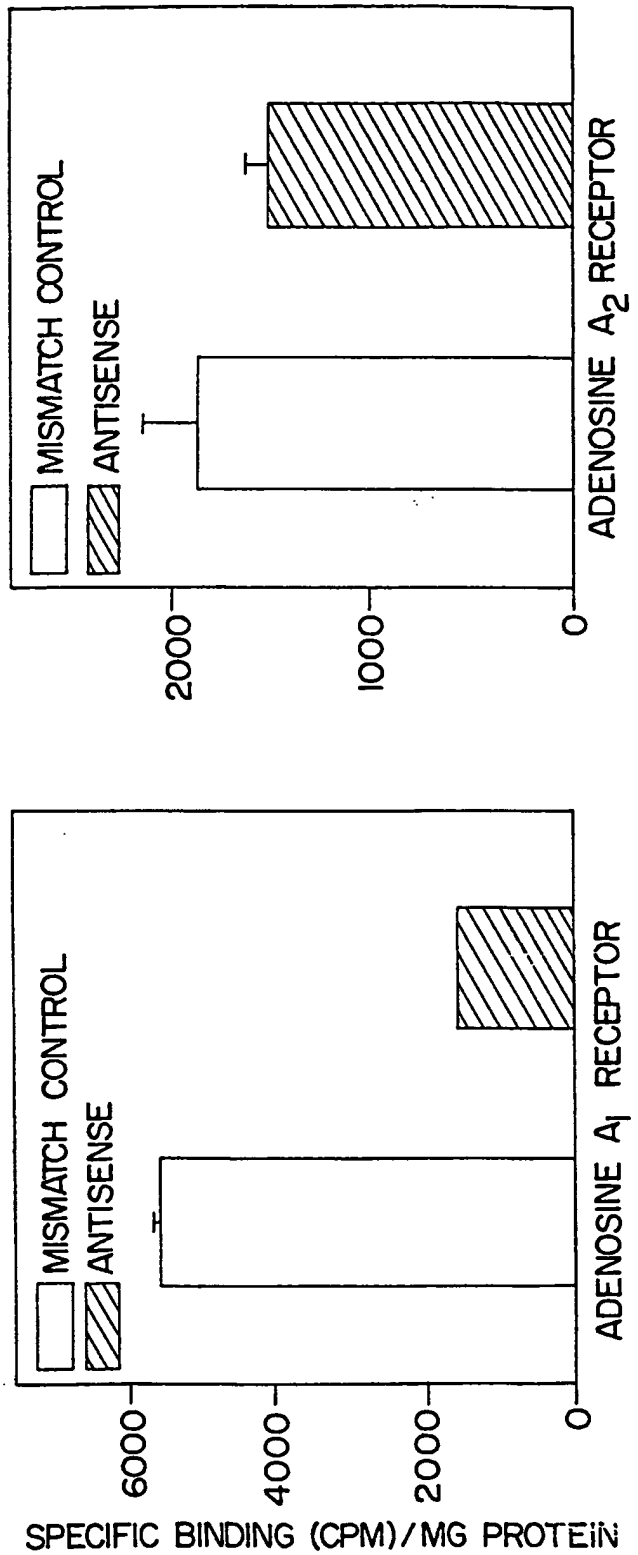
MISMATCH CONTROL
ANTISENSE
ADENOSINE A_2 RECEPTOR
2000
1000
0
ADENOSINE A_1 RECEPTOR
6000
4000
2000
0
SPECIFIC BINDING (CPM)/MG PROTEIN

FIG. 2.

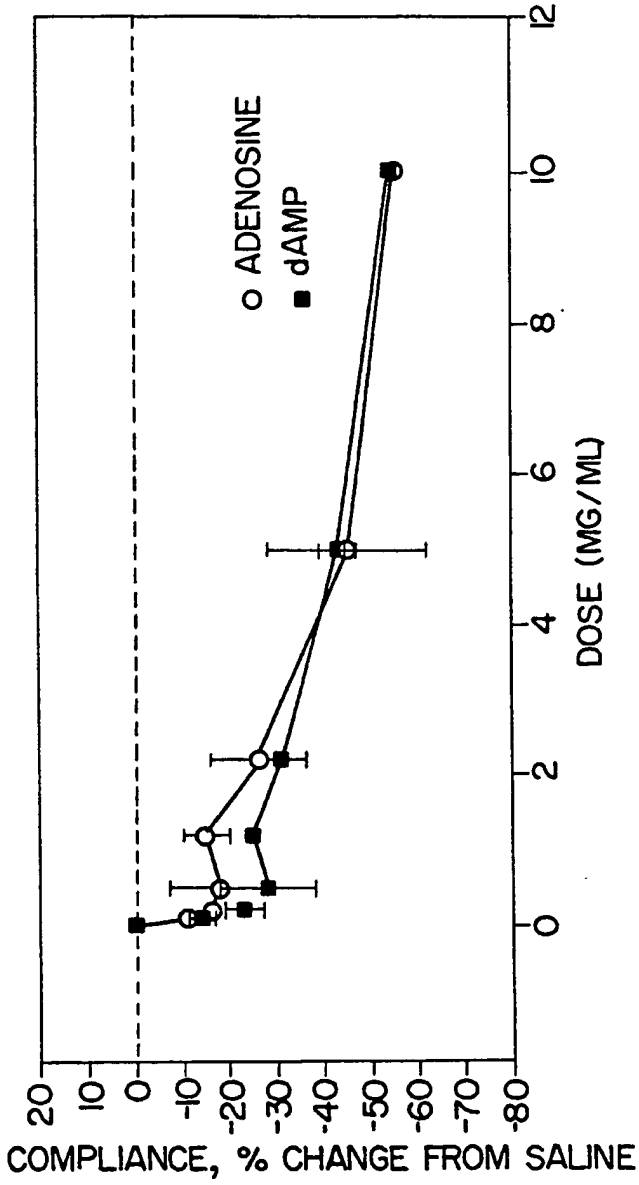
o ADENOSINE
■ dAMP
DOSE (MG/ML)
0
2
4
6
8
10
12
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
COMPLIANCE, % CHANGE FROM SALINE

FIG. 3.

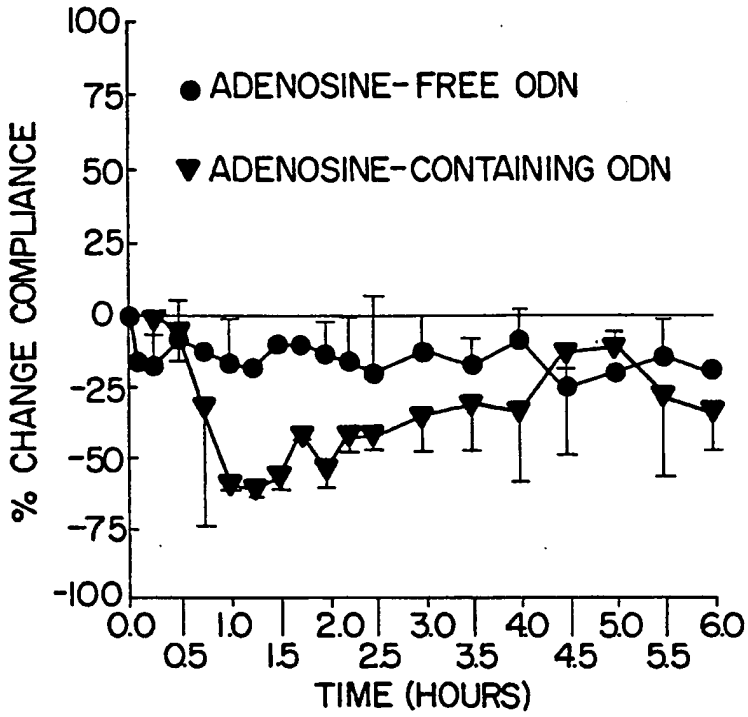
100
75
50
25
0
-25
-50
-75
-100
% CHANGE COMPLIANCE
● ADENOSINE-FREE ODN
▼ ADENOSINE-CONTAINING ODN
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
TIME (HOURS)

FIG. 4.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/09306

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/70
US CL :514/44; 536/23.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 5,514,788 A (BENNETT ET AL) 17 May 1993 (07.05.93), see entire document, especially Abstract, column 3, lines 15-18, column 5, lines 21-29, column 9, Figures 2 and 3.	1-6, 11-13, 15, 16 ---------- 7-10, 14, 17-20, 21
X -- Y	WO 94/02605 A1 (DUKE UNIVERSITY) 03 February 1994 (03.02.94), see entire document, especially page 5, lines 9-15, page 18, line 28, page 20, lines 2-5 , 11-15 and 31, page 21, lines 2-5 .	1-4, 6, 7, 9, 11-14, 16, 17, 19 ---------- 8, 10, 18, 20, 21
Y	US 5,264,618 A (FELGNER ET AL.) 23 November 1993 (23.11.93), see entire document, especially column 7, lines 40-42 and 54-56, column 8, lines 27-31, column 22, lines 12-15.	7-10, 17-20

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 18 AUGUST 1996	Date of mailing of the international search report 03 SEP 1996
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/09306

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KNIGHT, V et al. Antiviral therapy with small particle aerosols. European Journal of Clinical Microbiology and Infectious Diseases. December 1988, Vol. 7, No. 6, pages 721-731, Abstract only.	7-10, 17-20
Y	SCHREIER, H. The new frontier: gene and oligonucleotide therapy. Pharmaceutica Acta Helvetiae. January 1994, Vol. 68, No. 3, pages 145-159, Abstract only.	14
Y	US 5,521,291 A (CURIEL ET AL.) 15 December 1993 (15.12.93), see entire document, especially column 13, lines 49-54, column 25, lines 17-19, 46-50, 50-62.	21

INTERNATIONAL SEARCH REPORT

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B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

Medline, Biosis, Biotechds, Caplus, CJACS, Embase, Toxlit
Terms: (antisense or anti-sense); therap?; (lung disease or asthma or airway disease or bronchial?); adenosine; (cystic fibrosis or CF); liposome; (micron# or microm?); aerosol; Nyce J?/au; Metzger, w J?/au